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(*Recognosce notum, ignotum inspice*)

TRANSACTIONS

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NOTES ON ENTOMOGENOUS FUNGI

By T. PETCH

(With 8 Text-figures)

22. *Cordyceps bicephala* Berk.

This species was collected by Spruce at Panuré, Rio Negro, and was described by Berkeley in "Decades of Fungi," no. 617, *Hooker's Journ. Bot.* (1856), p. 278.

Berkeley described the head as elliptic. Cooke, *Vegetable Wasps and Plant Worms* (1892), p. 218, wrote "The heads are described as elliptic, but in the dried specimen they are as nearly as possible globose," and he gave a figure showing the stalk bifurcate above, each branch bearing a globose head. As previously pointed out by Lloyd (*Mycol. Notes*, no. 62 (1920), Fig. 1634), the heads are not globose. On the type specimen, they are ovoid, rounded or subacute above.

There does not appear to be any doubt that *Cordyceps australis* Speg. is the same species. Among numerous specimens collected by Mr Paul W. Richards in British Guiana, some have a lateral branch, a short distance below the head, which may remain sterile or may bear the conidial stage. Berkeley's specimen is exceptional in having two heads.

Mr Richards informs me that this species usually occurs on ants buried in moss on tree trunks, the insect being very much decayed. Berkeley's specimen was apparently gathered without the host insect. When fresh, the head and the upper part of the stalk are red, the remainder of the stalk being black. In formalin, the red parts become white, and in dried specimens they are white or pale ochraceous. The British Guiana specimens usually occurred on the ant, *Megaponera foetens*.

The conidial stage may arise, as stated above, as a branch of the perithecial clava, or as an independent linear clava from the host insect. This has been described by Spegazzini as *Isaria melanopus*. From Spegazzini's description and recent specimens, it is a *Hymenostilbe*, which must stand as ***Hymenostilbe melanopoda*** (Speg.), comb.nov. Spegazzini described the basidia (phialides) as cylindric, with an obtuse apex, 10–15 × 3 μ, and the conidia as obovate, 5–10 × 3–4 μ. On the British Guiana specimens, which had been preserved in formalin, the basidia were ovate, minutely verrucose at the apex, 7–9 × 4 μ, with a short central truncate sterigma, and the conidia hyaline, fusoid, with a truncate base, 7–9 × 1 μ.

In Herb. Kew. there is a specimen of *Cordyceps bicephala* from Uganda, R. A. Dummer, 4033, April, 1918.

From their descriptions, *Cordyceps proliferans* P. Henn., on *Dinoponera grandis*, Brazil, *C. Huberiana* P. Henn., on *Megaponera* sp., Para, and *C. necator* Pat. & Har., from French Guinea, do not appear to differ from *C. bicephala*.

23. *Cordyceps albella* Massee

This species was described by Massee in *Ann. Bot.* IX (1895), 39, from a specimen in Herb. Kew., collected by Wright in Cuba, which Berkeley had left unrecorded. Massee stated that it was on a grasshopper which was covered with white mycelium, and consisted of numerous white or yellowish slender clavae, 5 mm. to 1 cm. high, 1–2 mm. diameter, cylindric or claviform, arising from the lower surface of the thorax and abdomen.

In the specimen in its present condition, the clavae arise from a mass of frass, which is separate from the insect. The remains of the insect are in a separate packet, and do not show any white mycelium. It seems doubtful whether the fungus really grew on the insect.

The clavae are about 5 mm. high, terete, smooth, about 200 μ diameter below, attenuated upwards to 150 μ diameter, and then expanding into a long clavate head, about 360 μ diameter. They are composed of parallel agglutinated hyphae which separate into conidiophores above, with a few outlying conidiophores on the upper part of the stalk. The conidiophores are penicillioid, hyaline, with a stalk 2 μ diameter, either branched above, the branches terminating in cylindric or clavate phialides, 7–10 × 2–2·5 μ, or unbranched, bearing three similar phialides at the apex, each phialide bearing a chain of conidia. The conidia are hyaline, thick-walled, smooth, globose, 2–3 μ diameter, or broadly oval, 2·5–3·5 × 2–2·5 μ, catenulate, united by a distinct isthmus about 1 μ long. The conidiophores are closely packed together in the head, so that the head is compact, not loose.

This is a coremioid *Penicillium*, which must be known as *Penicillium* (*Coremium*) *albellum* (Massee), comb.nov.

A specimen from Ceylon, included in the cover of *Cordyceps albella* in Herb. Kew., is quite different from the Cuban specimen. It is either *Hirsutella* or *Hymenostilbe*, but is not in a condition for further determination. It appears to be on the remains of a firefly.

24. *Cordyceps palustris* B. & Br.

Cordyceps palustris was collected by Ravenel in South Carolina, and was described by Berkeley in *Journ. Linn. Soc.* 1 (1857), 159. There are specimens in Herb. Kew., "Ravenel 718, moist putrid logs, Northampton swamp," with a description of the colour, etc., from Ravenel,

and others, part of the same collection, in Herb. British Museum *ex* Herb. Ravenel. In both specimens the perithecia are globose, and the part-spores at first cuboid, becoming globose, 1·5 μ diameter. Berkeley described the perithecia as globose, and the part-spores as forming moniliform chains of globose grains.

Massee (*Ann. Bot.* IX (1895), 11) described and figured the perithecia as "cylindric, narrowed at the base when mature, and in reality quite superficial," and stated that he had not seen the spores broken up into part-spores.

Möller (*Phycomyceten und Ascomyceten*, p. 230) described a *Cordyceps* with globose part-spores as *C. hormospora*. It occurred in Brazil on beetle larvae in rotten logs. Möller stated that *C. hormospora* was very closely allied to *C. palustris*, the description of the latter in Cooke, *Vegetable Wasps*, etc., agreeing almost completely with the Brazilian species, but on the other hand the form and arrangement of the perithecia in *C. hormospora* were certainly widely different from the description given by Massee for *C. palustris*.

In actual fact, the perithecia of *C. palustris* are globose with a short neck, completely immersed, as described by Möller for *C. hormospora*, and they are arranged close together in a single layer immediately beneath the cortex. Massee must have examined some other species, perhaps *C. Ravenelii*. *C. hormospora* agrees completely with *C. palustris*, and the former name is consequently a synonym of the latter.

25. *Cordyceps dipterigena* B. & Br.

Cordyceps dipterigena, which grows on flies, was described by Berkeley and Broome in 1873. As previously stated by von Höhnel and myself independently, there is practically no doubt that *Torrubia coccigena*, described by the Tulasnes as occurring on a coccus, is the same species, the remains of the host having been misidentified.

Möller described it from Brazil as *Cordyceps muscicola*. I have hitherto been reluctant to accept this reference, as Möller's figure shows a different habit, six perithecial clavae arising from the insect, in place of the two perithecial and one conidial of the common Ceylon form. I have, however, recently examined a number of specimens from British Guiana and Panama, and find variation in the number and character of the clavae. One specimen from British Guiana (Richards, no number) has six perithecial clavae from the thorax, as in Möller's figure, with heads 2 mm. diameter, while another (Richards, 572) has two immature clavae from the thorax and a conidial clava from the tip of the abdomen, exactly as in the common Ceylon form. A specimen from Panama (Williams, 45) has two conidial clavae from the thorax, and two on the abdomen in the median line, while another Panama specimen (Williams, 46) has

two immature perithecial clavae from the tip of the abdomen. The conidial stage is the same as in the Ceylon specimens.

Most of the available Ceylon specimens were collected at a high elevation, and probably the smaller number of clavae is due to climatic causes. The heads of the perithecial clavae, however, vary from 1·2 mm. to 2·5 mm. diameter in the same gathering, and consequently the size of the head is not a character distinguishing the Ceylon from the American form.

Thwaites collected in Ceylon a specimen which bears nine immature perithecial clavae. The locality was not recorded, the specimen being accompanied by a note in Thwaites' handwriting, "collected 24 June 1861. Fungus on large dipterous insect for Rev^{d} M. J. Berkeley, with G. H. K. T.'s kind regards." It was most probably collected in the low-country. The specimen was not referred to in the *Fungi of Ceylon*. Lloyd named it *Cordyceps Thwaitesii*, but, as pointed out by Thaxter, it is immature *C. dipterigena*.

C. Ouwensii v. Höhnel, from Java, is a fairly frequent variation of *C. dipterigena*, and has been collected in Ceylon in company with the normal form.

C. Muscae P. Henn., from New Pomerania, is, from the type specimen, an immature clava of *C. dipterigena*.

C. opposita Syd., on a fly from New Guinea, is, from the type specimen, *C. dipterigena*. It has the two opposite perithecial clavae from the thorax, and the conidial clava from the abdomen, of the normal form. Sydow described and figured perithecia on the conidial clava. It is probable that these are the perithecia of the parasite, *Byssostilbe tomentosa*, as figured by me in *Trans. Brit. Myc. Soc.* VIII (1923), 211.

The synonymy of *Cordyceps dipterigena* is as follows:

Cordyceps dipterigena B. & Br. in *Journ. Linn. Soc.* XIV (1873), 111; *Torrubia coccigena* Tul., *Sel. Fung. Carp.* III (1865), 19; *Cordyceps coccigena* (Tul.) Sacc., *Syll. Fung.* II (1883), 576; *Cordyceps Muscae* P. Henn. in *Engl. Jahrb.* XXV (1898), 507; *Cordyceps muscicola* Möller, *Phycomyceten und Ascomyceten*, 221 (1901); *Cordyceps Ouwensii* v. H., *Fragm. z. Myk.* 214 (1909); *Cordyceps Thwaitesii* Lloyd, *Mycol. Notes*, 65 (1920), 1060; *Cordyceps opposita* Syd. in *Engl. Jahrb.* LVII (1922), 325.

Of the species of *Cordyceps* described as parasitic on flies, two appear to be valid, viz. *C. dipterigena* B. & Br. from the tropics, and *C. Forquignoni* Quélet from Europe. The name, *C. coccigena* (Tul.) Sacc., is a misnomer, and obviously to be discarded.

The conidial stage of *C. dipterigena* is a *Hymenostilbe*, which may be known as *Hymenostilbe dipterigena*. It usually arises from the tip of the abdomen, but may replace a perithecial clava from the thorax or arise from the broken stalk of a perithecial clava.

Hymenostilbe dipterigena Petch, n.sp. Clava terete, usually simple,

sometimes forked, up to 2 cm. long, 0·25 mm. diameter, pale brown becoming blackish brown, ashy above, surface matt, then pruinose; basidia cylindric or cylindrico-clavate, 9–20 × 2–5 μ, apex rounded, strongly verrucose or glandular in the upper half; conidia hyaline, smooth, clavate, cylindric or oval, 4–9 × 1·5–4 μ.

In the Ceylon form, the basidia are 14–18 × 2–3 μ and the conidia clavate or cylindric, 6–9 × 1·5–2 μ. In young specimens from Panama (Williams, 45 and 46) and Tobago (Williams, 47), the basidia are shorter and broader, 9–11 × 4–5 μ, the glandular processes are larger, up to 1·5 μ, and the conidia are clavate, 4–7 × 2 μ, or oval, 6–8 × 3–4 μ; but in a specimen from British Guiana (Richards, 572) the basidia are 18–20 × 3 μ, and the conidia 6–9 × 2·5–3 μ.

26. *Cordyceps Sphingum* (Schw.) B. & C.

Isaria Sphingum was described by Schweinitz in 1822. Link (1825) listed it as *I. sphingophila*. The Tulasnes (1865) described and figured the perfect stage, from American specimens, as *Torrubia Sphingum*, while Berkeley and Curtis enumerated it in 1869 as *Cordyceps Sphingum*.

As recorded by the Tulasnes, Lebert had previously (1858) described the perfect stage as *Akrophyton tuberculatum*. There is no doubt, from Lebert's figures, that he had the perithecial stage of *Isaria Sphingum*. Maire, in 1917, published the combination, *Cordyceps tuberculata* (Lebert) Maire, following the Brussels rule that the first trivial name applied to the ascigerous stage must be used. Lebert found his specimen in the Museum at Geneva. Its origin was not recorded, but the host insect was said to be *Sphinx pinastri*.

Cordyceps tuberculata is a fairly common and striking species, and has, in consequence, been frequently illustrated. It generally occurs on the perfect moth, but has also been found on caterpillars. It occurs on Micro-lepidoptera, as well as on the larger moths.

The perfect moth is found, sitting in a natural position on a tree trunk, or under a leaf, with the clavae radiating from it in all directions. The white or pale yellow mycelium covers the head, thorax, and abdomen, but if the moth sits with outspread wings it does not cover the wings. The mycelium, however, penetrates the wings along the larger veins. On moths which sit with folded wings the mycelium may cover the whole insect, sometimes entirely, sometimes leaving the tips of the wings visible; and the same effect is produced on insects which have fallen to the ground and possibly lost their wings.

Clavae are produced from the head, thorax, and abdomen, and along the veins of the wings in the case of moths which die with wings outstretched. If the insect has become a uniform mummy, clavae are produced anywhere.

The perithecial forms may be divided into three groups, which are connected with one another by all possible gradations, viz.:

A. Specimens with attenuated narrow conical clavae up to 2 inches long, which bear perithecia in groups on the clavae; this is the usual form on moths with extended wings.

B. Specimens with short stumpy columnar clavae, which bear a group of perithecia, usually at and on the apex.

C. Specimens without clavae, bearing perithecia directly on the mycelium which covers the insect.

The clavae have a central core of parallel hyphae, with a looser outer tomentose layer. When old, the outer layer is lost and the clavae appear horny. The perithecia are orange-yellow when fresh, but become red-brown when dry. Möller sent a specimen to Hennings, who described the perithecia on the clava as superficial, but Möller contradicted that and stated that they were immersed, sometimes to one-third their height. That is purely a matter of condition. If the clava has lost its outer tomentose coat, the perithecia are superficial.

In view of the existence of these different forms, it is not surprising that the fungus has received several names. The form usually figured is A. Berkeley and Curtis's type of *Cordyceps Sphingum* B. & C. is A. Forms B and C were figured in *Trans. Brit. Myc. Soc.* IX (1923), Pl. II, figs. 13, 14, as *Torrubiella ochracea* Pat.

Möller, in Brazil, sent form A to Hennings, who described it as *Cordyceps Moelleri*, while Möller himself described form C as *C. cristata*. *C. isarioides* Massee was based on a specimen in Herb. Kew., labelled with that name by Curtis; it consists of two clavae of form B, with fragments of the wing of a moth attached.

Isaria Sphingum was re-described by Vosseler in 1902 as *I. Surinamensis*, from a specimen from Surinam. He stated that he had submitted the specimen to Massee, who assigned it to *I. Sphingum*, but he did not accept that identification because its clavae were longer, up to 12 cm. Vosseler's figure, however, agrees completely with *I. Sphingum*. Vosseler found what he considered to be conidiophores on the clavae, but if so they were immature.

Vincens, in 1915, published an account of *Cordyceps Sphingum* (*Bull. Soc. Path. Végétale de France*, II, fasc. 1) and described the conidial stage in part. On a specimen which bore perithecia, he found conidiophores on minute clavae, not more than 2 mm. high, after searching for them in vain on the longer clavae. The conidial clavae had a conoid or cylindric base, contracted suddenly above into a slender appendage which bore a palisade layer of phialides. The phialides were figured as flask-shaped, bearing at their apex a chain of globose conidia, 2 μ diameter. Vincens' specimens were from South America.

In a collection of entomogenous fungi made by Mr C. B. Williams in Trinidad, British Guiana, etc., there are seven specimens of *Cordyceps tuberculata*. One of these is immature, the clavae just beginning to form. Another bears perithecial clavae only. A third bears perithecial clavae and conidial clavae, while the remaining four bear conidial clavae only. These conidial clavae, or synnemata, occur, scattered or crowded, on the mycelium on the head, thorax and abdomen, and in lines along the veins of the wings. They are about 0·5 mm. high and resemble small Stilbums. From a small conical base of interwoven hyphae, there arises a stem, about 50 μ diameter, which expands above into a globose or ovoid head, about 0·1 mm. diameter, white in colour. The stalk is not composed of parallel agglutinated hyphae as in *Stilbum*, but consists of hyphae which run vertically, but more or less irregularly, upwards. Externally it is either naked, or clothed with spreading hyphal ends. The hyphae branch above to form the head, in which they bear phialides terminally and laterally. These phialides are clavate, 9 μ high, 3 μ diameter above, the apex rounded. Each phialide bears a single chain of conidia, which are narrow-oval or cylindric, ends rounded or truncate, hyaline, continuous, 4–6 × 1–1·2 μ, becoming rather more oval, 1·5 μ broad. The conidia persist in chains for some time. In some cases, a long conidium appears to be produced, which subsequently divides into conidia of the normal length. When the synnema is mounted, the head appears densely fringed with radiating lines of conidia. Scattered phialides also occur on the hyphae of the stalk: in that situation they are conoid, or flask-shaped with a neck about 2 μ long, from 7 × 4 μ to 9 × 3 μ, bearing the same chains of conidia as in the head.

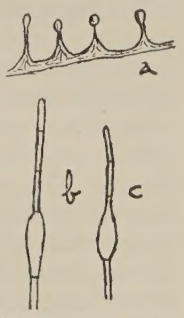

Fig. 1. *Isaria pistillariaeformis*. *a*, a row of synnemata, × 6; *b*, phialide and conidia from the head, × 1000; *c*, phialide and conidia from the stalk, × 1000.

The conidial stage in Williams' specimens is exactly *Isaria pistillariaeformis* Pat., described by Patouillard from specimens on a Micro-lepidopteron from Ecuador. It is apparently a common form in the West Indies, and is represented in a collection made by Miss Wakefield in that region. But, owing to the loose nature of the stalk, the heads are readily detached, and hence they are likely to be lost, unless the specimens are very carefully handled.

In a Ceylon specimen on *Psilogramma menophon* Cram., the well-known long clavae bear a palisade layer of phialides. These phialides are cylindric or clavate, rounded or acute above, 5–10 μ high, with a short sterigma up to 2 μ long. The conidia are oval or broadly oval, 3–4 × 1·5 μ. Towards the base of the clava, there are conidiophores up to 40 μ long, simple, or with one lateral branch, bearing

clavate conidia, 4–6 × 1·5–2 μ, in chains. In addition to the long clavae, this specimen bears short slender clavae, up to 2 mm. high, of uniform diameter, or suddenly attenuated to half the diameter at about half their height. These bear a palisade layer of phialides in the upper part, as described by Vincens, the phialides and conidia being the same as on the longer clavae of the specimen.

Another Ceylon specimen, on *Macroglossa affictitia*, also has a palisade layer of phialides on clavae, 2 cm. long. The phialides are same as in the foregoing specimen, but the sterigmata are up to 4 μ long, and the conidia are oval, 2·5–3 × 2 μ, or globose, 1·5–2 μ, with a mucous coat. A small clava, 0·6 mm. high, on this specimen, has the same phialides and conidia.

A third Ceylon specimen, on a micro-lepidopteron, has minute clavae, with phialides as above, sterigmata up to 3 μ high, and conidia cylindric, 7 × 1 μ, or oval, 5 × 1·5 μ, furnished with a mucous coat.

In *Trans. Brit. Myc. Soc.* x (1924), 43, I described *Hirsutella clavispora*, on a caterpillar attached to a living leaf. The caterpillar, which bore five long cylindric clavae, was covered with mycelium which extended from it to the leaf. A re-examination of this specimen, in comparison with more recent specimens of *Cordyceps tuberculata*, has convinced me that *Hirsutella clavispora* is a conidial form of that species. The conidia are cylindric or clavate, 4–8 × 1–1·5 μ, with a mucous coat. The sterigmata are only about 4 μ long, the previous longer measurement being probably based on a chain of conidia. These conidia agree with those of *Isaria pistillariaeformis*, but the latter have no mucous coat.

A further Ceylon collection, on caterpillars probably of *Ophiusa* sp. is immature *Cordyceps tuberculata*. Each caterpillar bears up to fifty clavae, about 1 cm. long, brown and subtranslucent towards the tip. They have passed the conidial stage, but a few phialides can be found. The clavae bear groups of perithecia in the upper half, which agree with those of *C. tuberculata*, but are immature. There is no covering of mycelium on the caterpillar.

Robin (*Végétaux Parasites*, Plate IX, fig. 6) gave a figure of a fungus on a caterpillar from the East Indies which is probably the conidial stage of *C. tuberculata*.

The conidial stage of *C. tuberculata* may take the form of a palisade layer of phialides on long clavae (*Isaria Sphingum*, *Hirsutella clavispora*), or a similar layer on a thin apical appendage of a minute clava about 2 mm. high, or a cluster of similar phialides forming the head of a minute *Stilbum* (*Isaria pistillariaeformis*). These forms may occur together on the same specimen, but the third has not yet been observed on specimens from the East Indies.

The variation in the conidia is considerable. In the *Isaria pistillariae-*

formis form the conidia are cylindric to oval. In *Hirsutella clavispora* or *Isaria Sphingum* forms, they are cylindric to clavate, or oval, or oval to globose, though the last are infrequent, and I have not seen chains of globose spores as figured by Vincens. It does not, however, appear possible to separate these forms as species.

The long clavae, which bear a palisade layer of phialides, should be classed as *Hymenostilbe* rather than *Hirsutella*.

The synonymy of this species is as follows:

Cordyceps tuberculata (Lebert) Maire in *Bull. Soc. d'Hist. Nat. de l'Afrique du Nord*, VIII (1917), 165; *Akrophyton tuberculatum* Lebert in *Zeitschr. f. wiss. Zool.* IX (1858), 448; *Torrubia Sphingum* (Schwein.) Tul., *Sel. Fung. Carp.* III (1865), 12; *Cordyceps Sphingum* (Schwein.) B. & C., *Fungi Cubenses*, no. 746 (1869); *Cordyceps Sphingum* (Tul.) Sacc., *Syll. Fung.* II (1883), 572; *Ophionectria Cockerellii* Ellis in *Journ. Inst. Jamaica*, I (1892), 142; *Cordyceps Cockerellii* Ellis, *op. cit*, p. 180; *Cordyceps isarioides* Curtis MS. *ex* Massee in *Ann. Bot.* IX (1895), 36; *Cordyceps Moelleri* P. Henn. in *Hedwigia* (1897), 221; *Cordyceps cristata* Möll., *Phycomyceten und Ascomyceten* (1901), 212; *Cordyceps rostrata* P. Henn. in *Hedwigia*, XLI (1902), 165; *Cordyceps tarapotensis* P. Henn. in *Hedwigia*, XLIII (1904), 246; *Torrubiella ochracea* Pat. in *Bull. Soc. Myc. France*, XXII (1906), 58.

Conidial stage: *Hymenostilbe Sphingum* (Schwein.) Petch, comb. nov.; *Isaria Sphingum* Schwein., *Syn. fung. Car. Sup.*, no. 1298 (1822); *Isaria sphingophila* Link, *Sp. Plant.* VI, 2 (1825), 114; *Isaria pistillariaeformis* Pat. in *Bull. Soc. Myc. France*, IX (1893), 163; *Isaria surinamensis* Vosseler in *Jahresb. d. Vereins f. vaterl. Naturk. in Würtemburg*, LXIII (1902), 380; *Hirsutella clavispora* Petch in *Trans. British Myc. Soc.* X (1924), 43.

Cooke, *Vegetable Wasps and Plant Worms* (1892) did not give Britain as a locality for *Isaria Sphingum*. Massee, *Fungus Flora*, III (1893), 447, recorded it "on the pupa of a dipterous insect in Scotland." That record is from Berkeley and Broome, *Notices of British Fungi*, no. 1710 (1878), and it is erroneous.

27. *Cordyceps Mawleyi* Westwood

The name, *Cordyceps Mawleyi* Westwood, was published by L. O. Westwood in *Gardeners' Chronicle*, IX, Ser. 3 (1891), 553, for a fungus found in numbers in a border of herbaceous perennials in a garden at Berkhamsted. They were said to occur on an underground caterpillar of either a Noctuid or a *Hepialus*, about one inch below the surface. No details of colour or size were given, and apparently all were immature, as no spores were mentioned. The fungus was said to produce a profusion of filaments from the segments behind the head of the caterpillar. The figure apparently shows an immature clava and strands of mycelium arising near the head. The figure

would pass well for an immature *Cordyceps gracilis* (Grev.) Dur. and Mont., and the host and habitat agree with that identification. In the absence of any distinguishing characters, the best that can be done for *C. Mawleyi* is to place it as a synonym of *C. gracilis*.

28. *Cordyceps Lloydii* Fawcett

This species was described and figured by Fawcett as having a single capitate clava, arising from between the head and the thorax of an ant. From specimens collected recently by Mr Paul W. Richards in British Guiana, it appears that it may produce two perithecial clavae, both dorsal, one between the head and the thorax, and the other between the thorax and the abdomen, each seated on a narrow white band of mycelium which encircles the body of the ant at the joint. The insect is attached to the leaf by pale brown mycelium. The free part-spores are elongated oval, ends truncate, 7–10 × 1–1·2 μ. The host in these examples is *Camponotus* sp.

Two of Mr Richards' specimens bear conidial clavae, arising in the same positions as the perithecial clavae. These are either simple and linear, or are stout below and branch into thin clavae above. It seems probable that the latter arise from decapitated immature perithecial clavae.

Of two specimens from British Guiana, collected by Mr A. W. Bartlett, one bears perithecial clavae and the other conidial clavae. The latter has a clava from between the thorax and the abdomen, which begins as an erect stout stalk about 0·15 mm. diameter, abruptly contracted above to 0·1 mm. diameter, and continued as a long thin flexuose appendage; and another, long, thin, flexuose, 0·1 mm. diameter, arising from the pad of mycelium beneath the thorax, which fastens the insect to the leaf.

This conidial stage is a *Hymenostilbe*, which may be known as *Hymenostilbe Formicarum*.

Hymenostilbe Formicarum Petch, n.sp. Clava terete, simple, up to 14 mm. long, 0·2 mm. diameter expanding to 0·3 mm. above, or with a stout stalk, up to 5 mm. high, forking into two long, thin, linear clavae above, pale brown, ashy above, fibrillose below, pruinose above; basidia cylindric, 24 × 4 μ, verrucose above, with one, or two, broad truncate sterigmata; conidia narrow-clavate or subcylindric, one end acute, the other truncate or rounded, 6–11 × 1–1·2 μ. The conidial stage of *Cordyceps Lloydii* Fawcett. British Guiana, Richards, no. 774, 238 (part); A. W. Bartlett.

I have recently received specimens of *C. Lloydii*, on *Polyrachis* sp., from Dr G. O. Ocfemia, Philippine Islands.

As previously recorded (*Trans. Brit. Myc. Soc.* xvi (1931), 75), *Cordyceps Sherringii* Massee is immature *C. Lloydii*.

Examination of the type specimen shows that *Cordyceps subdiscoidea* P. Henn. in *Hedwigia*, XLI (1902), 168, is *C. Lloydii*.

29. *Cordyceps* on Hymenoptera

Lebert, in *Zeitschr. f. wiss. Zool.* IX (1858), 452, instituted the genus *Polistophthora*, with the species, *Polistophthora Antillarum*, for a fungus on a wasp from Jamaica. Lebert stated that it was the "Vegetable Fly," and from his figures and description there is no doubt that it was *Sphaeria sphecocephala* Kl. in Herb. Hooker, Berkeley (1843), which is *Cordyceps sphecophila* B. & C. (1869). Lebert's genus and trivial name are consequently superfluous.

Cordyceps gentilis (Ces.) Sacc. was described by Cesati from a specimen on a wasp from Sarawak. I have recently examined a specimen on a hornet from Siam. The stalk was over 5 cm. long, 0·5 mm. diameter, pale brown, matt, not horny, longitudinally striate, and of the same texture as that of *C. sphecophila*, the outer layer tending to split and curve away, like the outer layer of the stalk in some agarics. The head was up to 12 mm. long, 1·2 mm. diameter, cylindric, acute at the apex with a short thin tip, yellow, glabrous, irregularly longitudinally ridged and furrowed. The part-spores were narrow-oval or sub-fusoid, 8–11 × 1·5–2·5 μ. The structure of the head and the arrangement of the perithecia were the same as in *C. sphecophila*.

C. gentilis differs from *C. sphecophila* in its longer, more acute head, which becomes irregular (? in drying). In the latter species, the head is ovoid or narrow-oval, and acute. *C. oxycephala* Penz. & Sacc., on a wasp from Java, which was described and figured with an oblongo-fusoid, acute head, and was said to differ from *C. sphecophila* in its more acute, rougher head, is evidently *C. gentilis*. There is very little difference between *C. sphecophila* and *C. gentilis*. In the type of *C. sphecophila*, the part-spores are narrow oval, 10–15 × 1·5–2 μ.

C. lachnopoda Penz. and Sacc., also on a hymenopteron from Java, was described as having a yellow stalk, covered everywhere with hyaline spreading hairs. It would be of interest to know whether these hairs are the remains of conidiophores. Except for that feature, the description and figure do not reveal any marked difference from *C. sphecophila*.

C. thyrsoides Möller was described from a specimen on a bee from Brazil. From the description and figure, it is *C. sphecophila*.

The parallel European species, *C. Ditmari* Quélet, was described from a specimen on a wasp found in Jura. It was figured by Quélet with a double collar on the stalk, just below the head, but he stated in his description, "la cuticule forme parfois, en s'exfoliant, une double collerette fimbriée," so that he evidently recognised that this feature was accidental. The stalk of *C. Ditmari* is of the same texture

as that of *C. sphecophila*, and in both species the outer layer may split off and recurve.

In the European specimens of *C. Ditmari* issued in Rehm, *Ascomyceten*, no. 1287, on *Vespa* sp., the head is somewhat one-sided, with a free lower edge, differing in the latter respect from *C. sphecophila*, in which the head merges below into the stalk. The surface of the head is yellow, smooth and waxy looking, and the cortical layer consists of long clavate cells fused laterally. In *C. sphecophila* and *C. gentilis*, the appearance of the surface of the head is the same, but the cortical layer in these two consists of merely cuboid cells. In all three, the perithecia are subvertical, and readily separable from the friable context. The part-spores of this specimen of *C. Ditmari* were narrow-oval, ends obtuse, 8–9 × 1·5–2 μ.

In the copy of Rehm, *Ascomyceten*, no. 1287, examined, there was a conidial clava. This was simple, terete, linear, bifurcating at the apex. It had a central core of parallel hyphae, with an exterior palisade layer of basidia. The basidia were 16–24 μ high, 4 μ diameter, clavate, with a truncate apex. The conidia were borne singly on each basidium, and were broadly clavate, with a rounded apex, hyaline, thick-walled, 7–9 × 3·5–4 μ. This conidial stage is a *Hymenostilbe*. The cortical cells of the head of the ascigerous stage resemble the basidia of the conidial stage.

Specimens of *C. Ditmari* were also issued in Krieger, *Fungi Saxonici*, no. 1228, on *Vespa vulgaris* Linn., but these are conidial only. The stalk is white, smooth and glabrous, and does not bear any basidia. It terminates in an ovoid or globose head, covered with a palisade layer of basidia. The basidia are 18 μ high, 2 μ diameter, equal, cylindric, septate, bearing single hyaline clavate conidia, with obtuse ends, 2·5–3 × 1·5 μ.

A conidial specimen in Herb. Kew., labelled "St Die (Vosges)," has irregular, laterally flattened heads, tuberculate at the edge and margined below. The head is pale yellow, and is covered by a palisade layer of basidia. The basidia are cylindric, obtuse, 12–18 μ high, 1·5 μ diameter, and the conidia hyaline, oval, or pyriform, obtuse below, 2·5–3 × 1–1·5 μ. This agrees with Krieger, 1228.

Assuming that all these specimens of "*C. Ditmari*" are really the same species, then it would appear that its conidial stage can occur in two forms, the one a simple linear *Hymenostilbe*, and the other a capitate clava, with basidia confined to the head, which perhaps becomes perithecial later. But if that is the case, the basidia and conidia of the capitate form are much reduced in size, compared with those of the linear clava. There is, of course, the alternative, that these conidial stages belong to two distinct species of *Cordyceps*.

Quélet stated that *Cordyceps Ditmari* had conidia 10 μ long, ellipsoid, at first continuous and "fauve," then one-septate and brown

There is little doubt that his description applies to some intrusive spore. Foreign spores readily adhere to the clava of a *Hymenostilbe*, or *Hirsutella*, etc., and I have examined a specimen of *Hymenostilbe muscaria*, in which the clava was covered with oval, dark brown spores, apparently the ascospores of a *Hypoxylon*.

The exsiccati, Rehm, 1287 and Krieger, 1228, were issued under the name *Cordyceps sphecophila* (Kl.) B. and C., with the synonym, *C. Ditmari* Quélet. This synonymy would appear doubtful. The stalks of the two species are similar, and the colour and surface of the head, and the arrangement of the perithecia are the same, but there are differences in the structure of the cortex and the shape of the head, more especially the definite lower margin of the head of *C. Ditmari*. *C. Ditmari* might rank as a variety of *C. sphecophila*. But until the conidial stage of the latter is known, it is best to leave the question open.

Quélet believed that *C. Ditmari* was the perithecial stage of *Isaria sphecophila* Ditm. Ditmar's figure of his species is an obstacle to the acceptance of this allocation, for he described the clava as pilose at the apex and figured it minutely setulose. As previously pointed out by Speare, Ditmar's figure strongly suggests a *Hirsutella*. It does not agree with a *Hymenostilbe*. But here again, more specimens are required, before any satisfactory conclusion can be reached.

30. *Hymenostilbe Aranearum* Petch, n.sp.

This species has been found repeatedly at Nuwara Eliya, Ceylon, where it usually occurs on spiders buried in moss or concealed in cracks in the bark of trees, not attached to the substratum. As many as forty clavae may arise from one insect.

Hymenostilbe Aranearum Petch, n.sp. Clavae terete, up to 4 mm. high, 0·1 mm. diameter, usually simple, sometimes forked, attenuated upwards, with a clavate apex, pruinose, brown below, ashy above; basidia clavate or cylindric, rounded above, attenuated below, about 8 μ high and 4 μ diameter, minutely verrucose above, with a single short obtuse sterigma, up to 0·5 μ high; conidia obclavate, rounded at the base, acute or obtuse at the apex, hyaline, smooth, 6–8 × 2–2·5 μ. On spiders, Nuwara Eliya, Ceylon.

In very wet weather, the clavae of specimens protected in cracks in the bark of trees appear stouter, owing to the copious development of conidia which then persist in chains, discernible under a low magnification.

31. *Isaria crassa* Pers.

Isaria crassa was described by Persoon in 1797, as "simplicissima, clavula crassa subconica, stipite distincto glabro." Link (*Species Plantarum*, 1825) regarded it as a form of *I. farinosa*, and that view has been generally accepted. Persoon cited, with a query, Fig. *a*

of Holmskiold's plate of *I. farinosa*. That figure shows a form with a slender stalk and a clavate, unlobed head. Payer, in *Botanique Cryptogamique*, gave an alleged figure of *I. crassa* (p. 58, Fig. 241 and p. 76, Fig. 353), but his illustration was taken from Ditmar's figure of *I. sphecophila*. Roumeguère also gave a figure of *I. crassa* in *Cryptogamie Illustrée* (Fig. 309), but this is a copy of Ditmar's figure of *I. velutipes* Link.

32. *Isaria Pattersonii* Massee

Isaria Pattersonii was described by Massee in *Kew Bulletin* (1912), 358, as "Stroma effusum, crustaceo-tomentellum, matricem totam externe atque interne ambiens, cinereum, ubique ramulos erectos multifidos emittens; ramuli ex hyphis filiformibus densissime constipatis apice plus vel minus liberis efformati; conidia in hypharum apicibus acrogena, numerosissima, globosa, hyalina, 2 μ diametro." It was said to be on a scale insect, *Nuzura viridula*, from the Gold Coast. In *Kew Bulletin* (1913), 105, the host and locality were corrected to "on a pentatomid," *Nezera viridula*, from St Vincent, and it was added that specimens had also been received from Grenada.

In the type specimen the synnemata are fasciculate, arising from a tuft or cushion of mycelium, greyish white, up to 2 mm. high, 0·1 mm. diameter, with a cylindric or ovoid farinose head, 0·5 mm. high, 0·25 mm. diameter. The stalk is laterally compressed, loose, composed externally of loosely interwoven hyphae, and loose internally, except in some cases a stouter core towards the base. The heads are sometimes laterally adherent. Some of the conidiophores separate from the stalk below the head, but the head is somewhat compact. The conidiophore is spicarioid, with a main stem, 2 μ diameter, expanding above to 4 μ, and bearing, at the apex of the terminal segment, three or more short branches, crowned with phialides, or prophialides and phialides. The prophialides are cylindric, 7 × 2·5 μ, and the phialides flask-shaped, 7–8 × 2·5 μ. The conidia are catenulate, oblong-oval or subcymbiform, ends obtuse, 2– 2·5 × 1 μ, or subglobose, 1·5–2 μ, hyaline, smooth.

This species should be compared with *Isaria edessicola* Speg. (1910), described from a specimen on a Pentatomid (*Edessa meditabunda*) in the Argentine, which was said to have elliptico-cylindric conidia, 2–3 × 0·75–1 μ.

35. *Hypocrella palmicola* P. Henn.

Hypocrella palmicola was described by Hennings from a specimen from Madagascar. When preparing my paper on "The Genera *Hypocrella* and *Aschersonia*" (*Ann. Perad.* VII, 167–278), I examined the type in Herb. Berlin, which contained only one stroma. The specimen was rather old, and was blackish brown, dotted with black

ostiola. The centre of the stroma was depressed, and surrounded by pycnidia which opened into the depression. The remainder of the stroma contained crowded perithecia.

Recently, I collected numbers of this species at Nuwara Eliya, Ceylon, generally on an Aleyrodid on bamboo (*Arundinaria debilis* Thw.), but occasionally on other plants, *e.g. Litsea*, *Eugenia*, etc. Old specimens resemble the type in colour, but the younger and less weathered specimens are quite different. The difference in colour is parallel to that in *Aschersonia brunnea* Petch, a species of similar structure, originally described from an old herbarium specimen as dark purple-brown, but found to be yellow-brown in recently collected unweathered specimens (*Ann. Perad.* VII (1922), 326). The following description of *Hypocrella palmicola* has been drawn up from the Ceylon specimens.

Hypocrella palmicola P. Henn. in Voeltzkow, *Reise Ost-Afrika*, III, (1908), 29. Stroma circular, up to 3 mm. diameter, flattened pulvinate, margin rounded, yellow-brown or brownish yellow, subtranslucent (when fresh), yellowish grey or greenish grey (when dry), becoming brown and finally purple-brown with age, pruinose, becoming glabrous, internally pale yellow. Stromata perithecial, or pycnidial, or both. Pycnidium either central, a depressed circular cavity about one-half the diameter of the stroma, or a horse-shoe groove, sometimes interrupted, running concentrically midway between the centre and the circumference; groove, in section, either widely open, 0·5 mm. broad above, 0·25 mm. deep, with a convoluted base, or perithecioid, 0·25 mm. deep, 0·2 mm. broad in the middle, contracted to a narrow opening, 0·05 mm. across. Pycnospores fusoid, inequilateral, or subcymbiform, ends obtuse or subacute, 5–7 × 1 μ, extruded into a central orange mass; paraphyses linear, up to 120 μ long, but sometimes only 40 μ. Perithecia crowded, flask-shaped, 0·1 mm. deep, 0·05 mm. diameter, wall yellow, ostiola not projecting, red-brown, becoming dark brown and ultimately not evident; asci 80–100 μ long, 3–4 μ diameter, four-spored; part-spores cylindric, ends obtuse, 6–10 × 1 μ. Madagascar, in Herb. Berlin. Ceylon, on an Aleyrodid on bamboo, etc., Nuwara Eliya, 1926–28.

In one gathering on *Eugenia* sp. from Nuwara Eliya, the Aleyrodid is surrounded by a marginal, light brown, swollen ring, sometimes extending outwards as a narrow byssoid hypothallus. On this ring are situated close-set tubercles, laterally compressed, dark brown, minutely tomentose or pruinose, laterally ovoid, sometimes confluent. In one example, these tubercles cover the insect completely, but in general, the insect is exposed in the centre of the stroma. Each tubercle contains a vertically ovoid pycnidium, 0·4 mm. high, 0·25 mm. diameter, with an impressed ostiolum. The pycnospores measure 6–8 × 1·5 μ, and the paraphyses are up to 80 μ long. Similar

stromata have also been found in company with normal stromata of *Hypocrella palmicola*, and they are no doubt abnormal forms of that species.

In "The Genera *Hypocrella* and *Aschersonia*," a gathering from Trinidad, made by Professor Thaxter, was referred to *Hypocrella palmicola*. In the light of these later Ceylon specimens, in better condition than the type, that reference was incorrect. The Trinidad specimens are a distinct species, differing, *inter alia*, in the shape of the pycnidia and perithecia.

34. *Hypocrella castanea* Petch, n.sp.

This species was collected by Professor R. Thaxter, on an Aleyrodid on *Adiantum* sp., St Ann's, Port of Spain, Trinidad, and was referred to *Hypocrella palmicola* P. Henn., in *Ann. Perad.* VII (1921), 237.

Hypocrella castanea Petch, n.sp. Stromata dark chestnut-brown, circular, discoid, minutely rugose, up to 1·5 mm. diam., 0·8 mm. thick in the centre, thinning slightly towards the margin, internally pallid or brownish. Perithecia moderately crowded, globose with a conical neck, 200–250 μ high, 110–130 μ diameter, ostiola not projecting; asci and spores of the genus; part-spores cylindric, ends obtuse, 5–7 × 1–1·5 μ.

Aschersonia stage: stromata of the same colour, circular, flattened pulvinate or subdiscoid, 0·8–1 mm. diameter, up to 0·6 mm. thick, even, or umbilicate in the centre; pycnidium single, central, laterally oval, 0·3 mm. diameter, 0·2 mm. high; pycnospores fusoid, ends not produced, 4–6 × 1·5 μ; paraphyses linear, up to 130 μ long. On an Aleyrodid on *Adiantum* sp., Trinidad.

Figs. 31 and 32, Plate III, vol. VII, *Annals R.B.G. Peradeniya*, are the *Hypocrella* and *Aschersonia* stages respectively of this species.

35. *Torrubiella alba* Petch, n.sp.

This species, on spiders on the living leaves of various plants, was collected at Nuwara Eliya, Ceylon, on several occasions during 1927–8. It forms a rather loose white weft over the insect and spreads out in a byssoid film over the leaf. The perithecia are scattered, or clustered in lines or patches, on the mycelium on the insect, and scattered over the film on the leaf. In the majority of instances, it is associated with a *Cephalosporium*, which is apparently the conidial stage, and the same *Cephalosporium* has been found occurring alone on spiders.

The perithecia are clothed almost to the apex with white mycelium. If the *Cephalosporium* is absent, the hyphae on the perithecial wall towards the apex are rigid, curled, and here and there verrucose, but when the *Cephalosporium* is present, these hyphae are less evident,

and the covering on the perithecium consists largely of the mycelium and conidiophores of the latter. *Acrostalagmus*, as well as *Cephalosporium* conidiophores are produced. The conidiophores occur in loose tufts, and in one example, a clava, 8 mm. high, 0·15 mm. diameter, bearing loose tufts of conidiophores, is present.

When these specimens were collected, it appeared possible to divide them into two species, one having perithecia with a hyaline apex, and the other having perithecia with a brown apex. The *Cephalosporium* occurred in many of the examples of the former, but in none of the latter. When perithecia from fresh examples of the latter were mounted, however, they did not show any colour in the apex, the wall in all cases being uniformly hyaline. In general, the perithecial wall is so thin that the asci and the paraphyses are clearly visible when specimens are mounted in water, but occasionally it is stouter and the asci are not so clearly seen.

In the dry state, the apex of the perithecium is pale to dark amber, or, in one specimen, dark brown. But the apex of the latter was noted as hyaline when collected. The perithecium wall in this specimen is now pale yellow.

It has not been found possible to maintain the suggested division into two species on the character of the perithecium or the presence of *Cephalosporium* conidiophores.

Raciborski described *Barya montana* from a specimen on a spider in Java. From his description, it is evidently a *Torrubiella*, and must be known as *Torrubiella montana*. Raciborski stated that the insect was covered with a floccoso-pulverulent white mycelium, which at first bore a white, stilboid conidial clava, 4·5 mm. high, 0·5 mm. diameter, and then elongated-oval, free perithecia. He did not describe the conidial stage.

The Ceylon species differs in the shape of the perithecia, and especially in their size and texture. The Javan species was described as having perithecia with a stout wall, and 750–900 μ long and 400 μ broad. In the Ceylon species the perithecial wall is membranous, and the height does not exceed 500 μ, most of the perithecia being about 250 μ high.

The Ceylon species has a small number of linear paraphyses. Raciborski stated that *Barya montana* lacked paraphyses. It is not, however, advisable to lay much stress on that difference, as paraphyses in *Torrubiella* are often overlooked. As the genus *Torrubiella* now stands, it includes species which have, and species which, according to the descriptions, lack, paraphyses. But as the paraphyses in *Torrubiella* are evanescent and usually atypical, it would merely cause confusion if an attempt were made to divide the genus on that basis.

It is not possible to assign the Ceylon species to *Torrubiella montana*, or to any of the other species of *Torrubiella* known to occur on spiders.

Torrubiella alba Petch, n.sp. Mycelium white, loose, covering the insect and spreading in a byssoid film over the leaf. Perithecia scattered or crowded on the insect, and scattered on the film on the leaf, superficial, flask-shaped with a short neck, or conoid, 140–500 μ high, 55–200 μ diameter, clothed with white mycelium almost to the apex; apex rounded, thickened, pale or dark amber (in dry specimens); perithecial wall usually thin and translucent, hyaline, sometimes thicker and pale yellow; paraphyses few, linear, 0·5 μ diameter, as long as the asci, often showing light and dark lengths, diffluent; asci cylindric, 3 μ diameter, up to 200 μ long, eight-spored, apex capitate; ascospores linear, as long as the ascus, 0·75 μ diameter, multiseptate, dividing into cylindric, truncate part-spores, 2–4 × 0·75 μ. On spiders, Nuwara Eliya, Ceylon.

The conidial stage may be known as *Cephalosporium Aranearum.*

Cephalosporium (*Acrostalagmus*) *Aranearum* Petch, n.sp. Mycelium white, loose, covering the insect and spreading in a byssoid film over the leaf. Conidiophores in loose tufts, sometimes on a clava, simple, conoid, continuous or one-septate, up to 28 μ high, 1·5 μ diameter at the base, tapering regularly upwards, or acrostalagmoid, up to 150 μ high, 2 μ diameter below, with two whorls of phialides, 16 μ long, 1·5 diameter below, slightly flask-shaped, tapering to the apex. Heads of conidia small, 4–8 μ diameter, usually 4 μ, persistent. Conidia narrow-oval or cymbiform, straight or slightly curved, ends obtuse, hyaline, continuous, 1·5–4·5 × 0·75–1·5 μ. On spiders, Nuwara Eliya, Ceylon, sometimes accompanied by *Torrubiella alba.*

36. *Calonectria pruinosa* Petch, n.sp.

This species was found at Nuwara Eliya, Ceylon, on a leaf-hopper on bamboo and other plants. Its conidial stage was recorded for Ceylon in *Trans. Brit. Myc. Soc.* x (1924), 44, as *Hirsutella floccosa* Speare, but a comparison with specimens of the latter from Trinidad, etc., has shown that identification to be incorrect.

Calonectria pruinosa Petch, n.sp. Mycelium covering the insect with a compact dark ochraceous stroma of interwoven hyphae and spreading in a byssoid radiating mat over the leaf; stroma minutely pulverulent; subjacent hyphae white. Perithecia on, or partly embedded in, the stroma, dark red, pruinose with dark ochraceous mycelium almost to the apex, conoid, 0·1 mm. diameter, ostiolum papillate. Asci 150 μ long, 6–10 μ diameter, narrow-clavate, eight-spored, spores at first obliquely uniseriate, then uniseriate, or uniseriate below and irregularly biseriate above; apex of the ascus solid, 8–18 μ long, 6 μ diameter, truncate, sometimes depressed in the centre, perforated by a narrow canal. Paraphyses linear, shorter than the asci. Ascospores fusoid, seven-septate, apex obtuse, lower end attenuated and aseptate for 6–10 μ, hyaline, 18–28 μ long, 5–6 μ

diameter, sometimes with a mucilaginous coat, 1 μ thick. On a leaf-hopper on bamboo (*Arundinaria debilis*), etc., Nuwara Eliya, Ceylon.

The mycelium bears *Hirsutella* phialides, fairly close together, about 8 μ apart, and very variable in shape. The perithecial stroma is coloured only superficially, the underlying tissue being white. Stromata which bear conidia only are at first arachnoid and greyish white, becoming compact, white, or pale to dark ochraceous, or greyish brown. In one conidial example the stroma was deep orange-yellow.

This species differs from *H. floccosa* in colour and in the shape of the conidia. It may be known as *H. versicolor*.

Hirsutella versicolor Petch, n.sp. Stromata on the insect at first arachnoid, greyish white, becoming compact, white, then pale or dark ochraceous, or greyish brown, or orange-yellow, spreading in a byssoid radiating mat over the leaf. Phialides lateral on the hyphae, simple, conoid or flask-shaped, 8–20 μ high, 2·5–3 μ diameter, attenuated above into a long sterigma, or ovoid, 6 × 3 μ, narrowed abruptly into the sterigma, or compound, up to 26 μ high, septate and bearing lateral phialides below, frequently with two or three sterigmata on one phialide. Sterigmata up to 20 μ long. Conidia adherent, soon separating, narrow-cymbiform or narrow-oval, ends acute, hyaline, 4–8 × 0·75–1·5 μ. Sessile globose pseudo-conidia, 3–4 μ diameter, also occur on the mycelium. On a leaf-hopper, Nuwara Eliya, Ceylon.

Like *Hirsutella floccosa*, this species does not form a clava. Its conidia soon separate, and may be found adhering to the mycelium.

37. *Byssostilbe tomentosa* Petch

Byssostilbe tomentosa, a fungus parasitic on *Cordyceps dipterigena*, was described in *Trans. Brit. Myc. Soc.* VIII (1923), 211. In further collections of parasitised specimens of *Cordyceps dipterigena*, the *Byssostilbe* has been accompanied by two other fungi which are most probably stages of it.

One of these forms white, even patches, consisting of crowded *Cephalosporium* conidiophores, on the *Cordyceps* or on the insect, the latter being, of course, permeated by the mycelium of the *Cordyceps*. In one example, in which the body of the insect has been broken, the *Cephalosporium* covers the exposed surface with an even white pile. In addition, slender, white, conico-cylindric clavae, up to 1·5 mm. high and 0·25 mm. diameter at the base, are produced, on the insect and on the clavae of the *Cordyceps*, sometimes arising at right angles to the latter. These white clavae have a solid central core, formed by parallel hyphae of the *Cephalosporium*, and bear *Cephalosporium* conidiophores laterally, perpendicular to the clava and forming a rather loose peripheral layer. The conidiophores are simple, 16–20 μ

high, 2 μ diameter near the base, flask-shaped and septate below, attenuated in the upper half or one-third. The conidia are oblong-oval or sub-fusoid, 3–4 × 1·5 μ, persistent in globose heads up to 8 μ diameter. The conidiophores and conidia on the clavae are identical with those which occur on the *Cordyceps* directly, and it is clear that the fungus can take both *Cephalosporium* and *Tilachlidium* forms.

With the foregoing fungus are minute, yellow, subtranslucent, columnar masses, which at first sight appear to be developing perithecia. They occur in groups, partly embedded in the pile of conidiophores on the insect or on the *Tilachlidium* clavae. On examination these prove to be masses of conidia. The yellow masses are up to 0·25 mm. high, 0·1 mm. diameter, or larger by confluence. The embedded base is concave below, sub-hemispherical, up to 200 μ diameter and 160 μ high, yellow, and consists of parallel hyphae which divide above and terminate in stout basidia, 2–3 μ diameter, septate, verrucose, rounded above, each furnished with a thin, central, conical sterigma, up to 10 μ long. The conidia are hyaline, continuous, oval, with acute ends, 2–2·5 × 1·5 μ. It would appear that this form should be placed in the Tuberculariaceae, in or near *Dendrodochium*.

Two other specimens, collected at Nuwara Eliya, have been referred provisionally to *Byssostilbe tomentosa*. Both are conidial only. One is on a caterpillar and the other, as far as can be determined, apparently on a fly. Whether the insects have been previously attacked by other fungi cannot now be decided. The *Tilachlidium* clavae are cylindric, white, up to 1 mm. high, 0·1 mm. diameter below, with a central core of parallel hyphae and a peripheral layer of *Cephalosporium* conidiophores which project irregularly from the clava. On the upper part of the clava, the conidiophores are simple, up to 24 μ high, stout, elongated conical, but in the lower part they are up to 54 μ high and irregularly branched. The core of the clava is fuscous externally, but it would seem probable that this darkening of the external layer of the core is due to weathering during an arrest of growth of the clava before the conidiophores were produced—a view which finds support in the fact that, on one clava examined, numerous uredospores were found adhering to the core of the clava, at the base of the layer of conidiophores. The conidia are narrow-oval or sub-fusoid, ends obtuse, 3·5–4·5 × 1·5–2 μ.

With these clavae are short, white, columnar protuberances, about 0·25 mm. in height and diameter, bearing at the apex a minute hyaline globule of conidia. These conidia are narrow-oval, ends acute, 2–2·5 × 1 μ. The hyphae of the column are parallel, curving outwards above, the outer terminating in rough clavate tips, while the inner terminate in conidiophores as in *Byssostilbe tomentosa*, the mass of conidia being partly sunk in the apex of the column.

No perithecia occurred with these latter specimens, and consequently their reference to *Byssostilbe tomentosa* is uncertain. The *Cephalosporium* conidiophores, and the colour of the masses of conidia, differ from those of *Byssostilbe tomentosa*, and it is possible that they may belong to another species of *Byssostilbe*.

38. *Empusa Lecanii* Zimm.

A disease of *Lecanium viride* occurs in Ceylon, South India and Java, and is known in South India as the "grey fungus" to distinguish it from the white fungus disease, caused by *Cephalosporium Lecanii*. Zimmermann, who investigated the disease in Java, attributed it to an *Empusa*, which he named *E. Lecanii*.

An account of this disease was published in *Trans. Brit. Myc. Soc.* xi (1926), 254–8, where it was recorded that the colour of the dead scale is due principally to the secondary growth of *Cladosporium* and other Dematiae. A fine hyaline mycelium was also found on the dead insect, and this bore conidia which resembled the sporangia of a *Pythium*.

I have recently received from Dr G. O. Ocfemia, Philippine Islands, specimens of an aphid, *Aphis sacchari* Zehnt. in a similar condition. The dead insects are covered with *Cladosporium*, and among it are fine hyphae, bearing sporangia similar to those figured on p. 257 (*loc. cit.*). Dr Ocfemia states that the disease has killed 80–95 per cent. of the aphids in insect-proof cages used in experiments on the transmission of the Fiji banana disease.

A comparison of the figures referred to above with those on Plate XVI of Thaxter's *Entomophthoreae of the United States* will show clearly that the supposed sporangia are the secondary conidia of an *Empusa*, which, from the details available, should apparently be referred to *E. Fresenii* Nowak.

39. *Verticillium*, *Cladobotryum* and *Calcarisporium*

In *Trans. Brit. Myc. Soc.* xi (1926), 251–4, I discussed the species which have been known as *Verticillium heterocladum* Penzig, and pointed out that it scarcely seemed possible that the fungus known under that name in Florida could be the same as the species described by Penzig from Italy, more especially as the former was figured with one conidium at the apex of each phialide, whereas Penzig particularly stated that his species had one to three conidia at the apex of a phialide. Penzig suggested that his species should rather be referred to the genus *Cladobotryum* Corda, because of the arrangement of the conidia.

I have since collected at Nuwara Eliya, Ceylon, a Hyphomycete which bears a close resemblance to *Verticillium heterocladum*, as described and figured by Penzig. It occurred in one instance on a froghopper on bamboo, together with an indeterminable fungus and a

Cladosporium, and in another instance on an insect wing attached to a leaf.

In the specimen on a frog-hopper, the fungus produces conidiophores on the insect, and runs widely over the leaf, on which the insect is seated, in single hyphae which bear erect, rigid conidiophores, scattered, or sometimes clustered in small white tufts. The conidiophores are up to 320 μ high, 2 μ diameter below, with nine whorls of phialides in the upper 120 μ, but usually about 120 μ high, 1·5 μ diameter below, with whorls of phialides, 10–13 μ apart, and sometimes solitary phialides below. The phialides are up to five in a whorl, narrow flask-shaped, 5–8 μ long, 1–1·5 μ diameter below, and bear minute cylindrical sterigmata, usually simple, sometimes bifurcate, towards the apex. In some cases the apex is slightly inflated and the sterigmata radiate from the apex only. The conidia are oval- or oblong-oval, sometimes slightly attenuated to one end, sometimes acute below and occasionally apiculate, 3–5 × 1·5–2 μ, not persistent on the phialide.

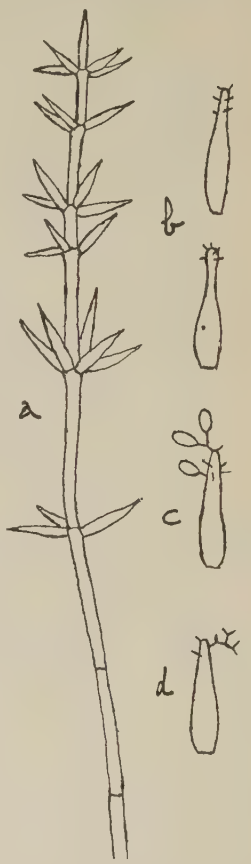

Fig. 2. *Cladobotryum ovalisporum*. *a*, conidiophore. × 1000; *b*, phialides; *c*, phialide with conidia; *d*, phialide with branched sterigma. × 2000.

In culture on Quaker Oat agar, the slant is covered with a rather dense white felt of mycelium and conidiophores, which becomes pinkish in the centre. The agar is not coloured. The sterigmata in culture are often elongated and branched.

In culture on Dox agar, the fungus produces compact, circular, flattened pulvinate stromata, at first pale purple-brown with a flat, white, tomentose margin. The reverse is then yellow. The stromata then become dark purple-brown towards the margin, and the reverse ochraceous. Finally, adjacent stromata coalesce and cover the whole slant, and become white owing to the development of conidiophores everywhere. The agar is not coloured.

This Ceylon species agrees with Penzig's *Verticillium heterocladum* in its verticillate phialides, and in having several conidia at or near the apex of a phialide. Neither is a typical *Verticillium*, but both have affinities with *Cladobotryum* and *Calcarisporium*.

On the other hand, the Florida species assigned to *Verticillium heterocladum* is, in the specimens I have examined, a true *Verticillium*.

The genus *Cladobotryum* was instituted by Nees in 1816 as conidiophore erect or decumbent, almost corymbosely branched above, with large oval or cylindrical spores round the apex of each branch, white. After stating that *Botrytis agaricina* and *B. macrospora* Link should be placed in this new genus, he described and figured *Cladobotryum varium* Nees. His figure shows a branched decumbent conidiophore, the branches bearing one to three conidia at the apex.

Fries (*Systema*, III (1832), 414) discarded Nees' genus, and placed *Botrytis macrospora*, *B. agaricina*, and *Cladobotryum varium* in the genus *Dactylium* Nees, regarding *Cladobotryum varium* as a form of *Botrytis macrospora*.

Thus, owing to Nees' unfortunate inclusion of two old species (with one of which he was admittedly unacquainted) in his new genus, and the consequent implication that his new species was similar to them, it might be considered necessary, in a complete treatment of the subject, to discuss *Botrytis agaricina* and *B. macrospora* —whether these two really have septate conidia, and whether they should be classed as *Verticillium*, or *Dactylium*, or *Diplocladium*, questions which involve citation from the mycological literature of more than a century. As, however, I propose to take *Cladobotryum* Nees as a genus having continuous spores, I have discarded four pages of manuscript dealing with these points. Nees apparently referred these two species to his new genus, because of the arrangement of their conidia.

Corda adopted Nees' genus *Cladobotryum*, and stated (*Ic. Fung.* I (1837), 21) that the merging of *Cladobotryum* in *Dactylium* was in the highest degree unnatural, because the latter genus had septate spores. But Corda emended Nees' description in such a manner that it may be taken to mean something quite different from Nees' idea, as expressed by his description and figure.

Saccardo (*Michelia*, II (1880), 18) again emended the generic description of *Cladobotryum*, and remarked (*Sylloge Fungorum*, IV, 161) that *Cladobotryum varium* Nees (which was Nees' type species) scarcely belonged to the genus!

Thus, *Cladobotryum* Nees, *Cladobotryum* Corda, and *Cladobotryum* Saccardo are three different things.

As I read Nees' description, *Cladobotryum* should have verticillately branched conidiophores with phialides in whorls, the phialides bearing apical and subapical sterigmata which bear solitary, continuous conidia. The phialide terminates in a slender apiculus (Corda), only when the terminal conidium has fallen off, leaving the short slender sterigma, while the later formed conidia are produced from similar sterigmata on the phialide, not at the base (Corda) or apex of the apical sterigma, and they are not "*aegre secedentia*" (Saccardo).

Another possible genus for Penzig's species is *Calcarisporium* Preuss,

which was instituted by Preuss in *Linnaea*, XXIV (1851), 124, and described as "Stipes erectus, septatus, hyphopodio ramoso suffultus, supra ramosus apice capitato verrucosus; verrucis sporas solitarias gerentibus. Sporae simplices basi hilo instructae." His single species was *Calcarisporium arbuscula*, and only one other (which does not wholly comply with the generic description) has been included in the *Sylloge Fungorum* up to the present time. The conidia, according to the generic description, should be beaked at the base, the name of the genus being based on that feature. One would expect that this refers to the conidia after abscission, not to the conidia while still attached to the phialide.

Calcarisporium arbuscula was re-described and figured by Miss A. L. Smith in *Trans. Brit. Myc. Soc.* III (1909), 120, Pl. VI, fig. 7, *a*, *b*, from specimens on decaying fungi from Swarraton, Hants. The figure shows somewhat flask-shaped phialides with a globose head which is rough with the points of attachment of the conidia, and the description states that the conidia have "sometimes a minute beak at place of insertion."

In "Studien ueber Hyphomyzeten" (*Centralb. f. Bakt.*, etc., Abt. 2, LX (1924), 1–26) von Höhnel stated that it was questionable whether *Cladobotryum* Sacc. (*non* Nees) was sufficiently distinct from *Calcarisporium* Preuss. He had collected on two occasions a Hyphomycete which he considered to be *Calcarisporium arbuscula* Preuss. It differed generically from *Verticillium*, only in having several conidia at the tips of the phialides, and in the tips of the phialides not being simple, but inflated into a head, about 3·5 μ broad, covered with stellately arranged, pointed warts, on which the very shortly stalked conidia were situated. On well-developed specimens, the phialides bore in addition a number of these warts below the head, so that a short spike of conidia was produced. On poorly developed specimens, the head was lacking and the phialide bore only one or two conidia at the apex. The conidia were hyaline, continuous, oval, pointed below, with a very short and thin pedicel.

It would appear from these descriptions that in *Calcarisporium arbuscula* the apex of the phialide may be capitate or not, while the conidium may or may not be beaked. It is not clear from these accounts whether the pedicel or sterigma remains attached to the spore after abscission or not.

Von Höhnel does not appear to have published any views on the genus *Cladobotryum*, and consequently one is at a loss to know why he considered that *Calcarisporium* was equivalent to *Cladobotryum* Sacc., not to *Cladobotryum* Nees. It does not answer to Saccardo's generic description, unless the conidia remain attached to the phialide.

Verticillium heterocladum Penzig and the corresponding Ceylon species both have phialides in whorls on the main axis, and one or more

conidia at the apex of a phialide. In the Ceylon species, the conidia are attached by short sterigmata, which persist on the phialide, the conidia, as a rule, not being beaked. Penzig stated that in *V. heterocladum* the conidia were generally borne on very short pedicels, but he did not describe them as pedicellate or beaked. The phialides in the Ceylon species are not, as a rule, inflated at the apex.

It would appear, however, that neither the inflated apex of the phialide, nor the presence of a beak or pedicel on the detached spore, —features which characterise the genus *Calcarisporium*—is constant. *Calcarisporium* Preuss should therefore be considered equivalent to *Cladobotryum* Nees. But even if that is not admitted, *Verticillium heterocladum* and the corresponding Ceylon species should be placed in *Cladobotryum* Nees.

The Florida fungus attributed to *Verticillium heterocladum* is certainly not that species. In the specimens from Florida which I have examined, it is a typical *Verticillium* with solitary terminal conidia on the phialides and no warts or sterigmata. It also differs in having some of the whorls of phialides on short lateral branches, whereas *V. heterocladum* (from Penzig's figure and description) and the Ceylon species have phialides arising in whorls from the main axis.

The following names are proposed for the species considered in this note:

Verticillium cinnamomeum Petch, nom.nov.; *Verticillium heterocladum* Fawcett *non* Penzig, *Fungi parasitic on Aleyrodes citri*, June, 1908. Stromata cinnamon-brown becoming ashy brown, pulverulent, flattened pulvinate, up to 2 mm. diameter and 0·6 mm. thick, compact, with a byssoid or strigose margin which merges into a thin, whitish, byssoid or pulverulent film extending over the leaf. Conidiophores arising on the stroma or on the film, hyaline, erect, slender, up to 240 μ high, 2–3 μ diameter, bearing in the upper part whorls of phialides or of short branches which bear phialides at the apex; lateral branches cylindric, 6–9 μ long, 2 μ diameter; phialides elongated-oval, attenuated above, somewhat inequilateral, curving upwards, 6–12 μ long, 2 μ diameter; conidia terminal, solitary, narrow-oval, hyaline, continuous, 3·5–6 × 1·5–2·5 μ. On *Aleyrodes citri*, etc., Florida.

Cladobotryum heterocladum (Penz.) Petch, comb.nov.; *Verticillium heterocladum* Penzig in *Michelia*, II (1882), 462.

Cladobotryum ovalisporum Petch, n.sp. Conidiophores borne on the host insect and on repent hyphae extending over the leaf, erect, scattered or clustered, hyaline, 120–320 μ high, 1·5–2 μ diameter below, with whorls of phialides in the upper half, and sometimes solitary phialides below; phialides up to five in a whorl, narrow flask-shaped, 5–8 μ long, 1–1·5 μ diameter below, bearing minute cylindrical sterigmata, sometimes forked, towards the apex; conidia

oval or oblong-oval, sometimes slightly attenuated to one end, sometimes acute below and occasionally apiculate, hyaline, continuous, not persistent on the phialide, 3–5 × 1·5–2 μ. On a frog-hopper, together with an indeterminable fungus, Nuwara Eliya, Ceylon, January 2nd, 1927.

This species apparently differs from *Cladobotryum heterocladum* in its smaller spores, but I have not seen a specimen of the latter. The details given by Penzig are insufficient for a closer comparison.

40. *Cephalosporium* on *Aleyrodes*

In *Trans. Brit. Myc. Soc.* x (1925), 154, *Cephalosporium Lecanii* Zimm. was recorded as occurring on a black Aleyrodid in Ceylon. During 1927–8, three more collections of this fungus on Aleyrodids were made at Nuwara Eliya, Ceylon.

Cephalosporium longisporum Petch, described from specimens on *Icerya purchasi*, Ceylon, has been found on an Aleyrodid on *Ocotea leucoxylon* (Sz.) Mez., Maricao, Porto Rico, collected March 23rd, 1916.

Neither of these species agrees with the description of *Cephalosporium Lefroyi* Horne, found on *Aleyrodes vaporariorum* at Wisley, England.

41. *Cephalosporium* on *Thrips*

Cephalosporium (*Acrostalagmus*) *Thripidum* Petch, n.sp. Mycelium covering the insect with a loose white weft. Simple conidiophores conical, up to 20 μ high; *Acrostalagmus* conidiophores up to 160 μ high, with one or two whorls of phialides towards the apex; phialides narrow flask-shaped, up to 24 μ long. Heads of conidia persistent, 8–14 μ diameter; conidia oblong-oval, ends obtuse, 3–6 × 1–2 μ. On *Thrips* spp., Nuwara Eliya, Ceylon, July 22nd, 1928, etc.

On Quaker Oat agar, the growth is white, thin and fleecy, and the agar is slightly reddened. On Dox agar, the growth is white, with a pale yellow reverse, and the agar is not coloured.

42. *Cephalosporium indicum* Petch, n.sp.

This species was sent to me in September, 1924, by Mr S. Sundararaman in culture from caterpillars on *Indigofera*, Mooply Valley, South India. Subsequently I received it from Mr C. S. Misra, of the Indian Lac Research Institute, Namkum, Ranchi, May, 1926, on larvae of *Eublemma amabilis*.

The larva is covered with a white felt of mycelium, the hyphae being 2·5–3 μ diameter. The conidiophores are lateral, scattered along the hyphae, sometimes opposite, either simple or branched. In nature, the simple conidiophores are elongated flask-shaped or elongated conoid, 8–12 μ high, 2·5 μ diameter below, but in culture they may be up to 50 μ high; sometimes they are one-septate. The

branched conidiophores usually have a single lateral branch, arising below a septum. Abnormal forms are common in culture; these have an inflated base, one or two-celled, up to 20 μ high and 7 μ diameter, from the apex of which arise several irregular conidiophores, which branch sympodially, the successive apices of the conidiophore being left as short conical projections. The total height of these irregular forms is up to 65 μ. The conidia form a loose head and soon separate; in nature they are hyaline, continuous, oblong-oval, one end usually acute, 4–9 × 2–2·5 μ; in culture, they are oblong-oval, usually attenuated towards one end or subfusoid, rounded at the apex, truncate at the base, 5–10 × 1·5–2·5 μ. The heads of conidia in hanging drops of Quaker Oat agar are oval, about 10 μ high and 6 μ diameter; sometimes the conidia occur in short chains, attached, not merely adherent. One conidium was seen in culture, which measured 12 × 2 μ and was one-septate.

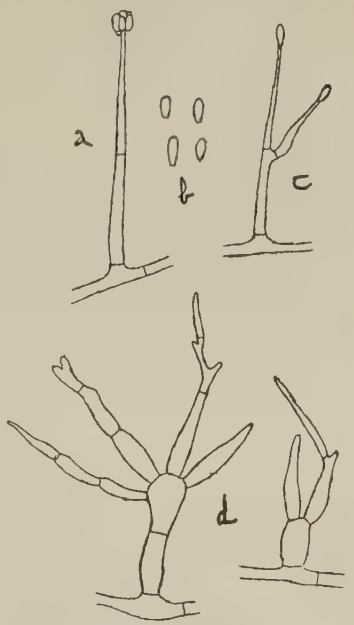

Fig. 3. *Cephalosporium indicum*. *a* and *c*, conidiophores; *b*, conidia; *d*, abnormal conidiophores from culture. × 650.

In culture on Quaker Oat agar, the fungus fills the tube with a white fleecy mass of mycelium, the conidiophores tending to be grouped in tufts; the agar is coloured salmon pink at first, becoming pale purple. On Dox agar the growth is of the same character, but the agar is not coloured; the reverse is cream-coloured.

Both on Quaker Oat agar and on Dox agar, minute black points appear at the edge of the slant in contact with the glass. These are minute brown or black-brown sclerotioid bodies.

The entomogenous species of *Cephalosporium* hitherto described have "persistent" heads, *i.e.* the conidia usually remain adherent for a considerable time. In the present species, the conidia soon separate.

Cephalosporium indicum Petch, n.sp. Mycelium 2·5–3 μ diameter, covering the insect with a white felt. Conidiophores lateral, scattered, elongated flask-shaped or elongated conoid, 2·5 μ diameter below, simple, 8–12 μ high, or branched, up to 20 μ high, with a single lateral branch; head oval, 10 × 6 μ, soon separating; conidia hyaline, continuous, oblong-oval, one end usually acute, 4–9 × 2–2·5 μ. On larvae of *Eublemma amabilis*, Namkum, India.

43. *Cephalosporium zeylanicum* Petch, n.sp.

This species is similar to *Cephalosporium indicum* in type of conidiophore and head, but differs from that species in its conidia and its behaviour in culture. On Quaker Oat agar, the mycelium covers the slant with an even, white, compact tomentose layer and the agar is not coloured. On Dox agar, it forms small scattered tufts which are ultimately united by a thin subtransparent layer, white and pulverulent; the reverse is cream, becoming buff, and the agar is not coloured.

Cephalosporium zeylanicum Petch, n.sp. Insect covered by a thin yellow film of mycelium, over which are scattered minute white tufts. Conidiophore simple, elongated conoid, 12–16 μ high, 2 μ diameter below, continuous, occasionally one-septate, or sometimes branched, up to 24 μ high, with a single lateral branch; conidia narrow-oval, both ends acute, hyaline, continuous, 3–4 × 1·5 μ. On a leaf-hopper, Nuwara Eliya, Ceylon, October 24th, 1926.

44. *Torrubiella hemipterigena* Petch, n.sp.

This species was common at Nuwara Eliya, Ceylon, during 1926–8, on a leaf-hopper, usually on bamboo (*Arundinaria debilis* Thw.), but occasionally on *Psychotria* and other plants. Most of the specimens bear only the conidial stage, which is a *Verticillium*.

Torrubiella hemipterigena Petch, n.sp. Insect covered by a dense yellow weft of mycelium, which spreads out in a whitish or greyish film over the leaf; sometimes with an erect yellow tomentose clava, cylindric, tapering upwards, up to 9 mm. high, 0·5 mm. diameter, arising from the anterior end of the insect. Mycelium of two kinds, pale lemon-yellow, 2–2·5 μ diameter, minutely spinulose, with lateral, sessile, oval, rough, spore-like bodies, 4–7 × 2·5–5 μ, singly below a node, and smooth, hyaline, 1·5 μ diameter, bearing conidiophores. Perithecia scattered, superficial, on the stroma on the body of the insect, flask-shaped, 0·8 mm. high, 0·3 mm. diameter below, with a cylindrical neck, 0·2 mm. high, 0·05 mm. diameter, clothed with a thin layer of yellow tomentum up to two-thirds their height, apex translucent amber. Asci 750 μ long, eight-

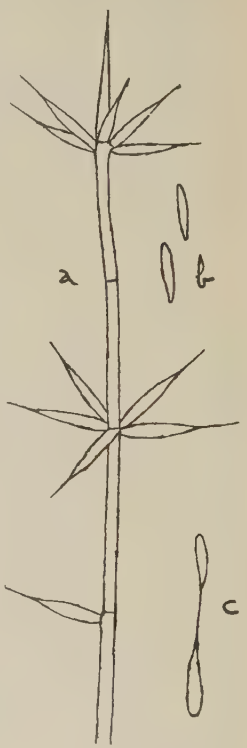

Fig. 4. *Verticillium hemipterigenum*. *a*, conidiophore; *b*, conidia; *c*, phialide with conidium. × 1000.

spored, capitate, 4 μ diameter; ascospores linear, 1 μ diameter, as long as the ascus, with septa, 8–13 μ apart; mature part-spores not seen. Conidiophores (*Verticillium hemipterigenum* Petch, n.sp.) on the stroma, the film on the leaf, the clavae, and the perithecia, scattered or clustered, up to 150 μ high, 1·5 μ diameter, with whorls of phialides in the upper part. Phialides up to six in a whorl, elongated flask-shaped, attenuated into a long thin tip, 12–16 μ long, 1 μ diameter below; conidia narrow-oval, or fusoid, or sub-falcate, ends acute, hyaline, continuous, 5–8 × 1 μ, usually solitary, occasionally two in a parallel bundle. On a leaf-hopper on bamboo (*Arundinaria debilis*), etc., Nuwara Eliya, Ceylon.

The grey film spreading over the leaf is composed partly of hyaline hyphae, with rough, lateral, spore-like bodies, like those on the yellow mycelium on the insect, and partly of the finer, smooth hyphae bearing conidiophores. Some specimens have a clava, others bear perithecia but no clava, while others have both.

On dried herbarium specimens, the perithecia appear dark red.

45. *Spicaria*

In *Trans. Brit. Myc. Soc.* x (1925), 186, 187, I described a Ceylon species of *Spicaria* on a spider, which I attributed to *Spicaria Araneae* Sawada.

Through the kindness of Professor Ito, I have received a translation of the description of *Spicaria Araneae*, which was published in Japanese, from which it appears that the Ceylon species is not the same as that described by Sawada from Formosa. The description of the latter is as follows:

"*Spicaria Araneae* Sawada, *Bot. Mag.*, Tokyo, xxviii, no. 331, p. (309), 1914. Body of the insect covered with a purple-grey cottony mass, not extending to the legs. Hyphae hyaline at first, pale brownish grey when old, septate, 4–5 μ diameter. Conidiophores hyaline, septate, about 500 μ high, 3–4 μ diameter, simple or rarely branched, with three to five whorls of prophialides and phialides towards the apex. Phialides flask-shaped, 7–8 × 2–2·5 μ. Conidia catenulate, long ellipsoidal or short cylindrical, ends rounded, hyaline, with two guttae, 3·5–5·5 × 2–2·5 μ.

"On the dead body of a spider, Formosa. Differs from *Spicaria prasina* (Maubl.) Saw. in colour, stouter conidiophores and shape of conidia."

Subsequently, I collected another coloured *Spicaria* on a spider at Nuwara Eliya, Ceylon, which differs from the previous Ceylon specimens in colour and in its larger phialides and conidia. The two Ceylon species resemble the Formosan species in their stout coloured mycelium, but differ from the latter in habit. *Spicaria Araneae* Saw. is said to cover the insect with a purple-grey cottony mass; in the

two Ceylon species the conidiophores form a dense even pile over the body of the insect, so even that at first sight one is liable to overlook the fact that the insect is covered by a fungus. In one of these Ceylon species the fungus is rose or purple-rose, becoming rose-purple when dry; in the other it is purple-grey to ashy.

All the Ceylon specimens differ from *Spicaria Araneae* in the shape of the phialides, which in the latter are described as flask-shaped. In dealing with other tropical species of *Spicaria*, it has been found that the shape of the phialides is a fairly constant character by which species may be differentiated, and consequently it is considered that this difference, taken with other characters, justifies the description of the Ceylon fungi as new species.

Spicaria velutiformis Petch, n.sp. Bright rose or purple-rose, becoming rose-purple when dry. Mycelium stout, 3–5 μ diameter, purple-brown. Conidiophores erect, crowded, covering the insect with a dense pile, about 100 μ high, stout, 3–4 μ diameter, septate, with several whorls of prophialides and phialides, 16–25 μ apart; prophialides oblong-oval or clavate, 4–5 × 2–3 μ; phialides cylindric, narrow-oval or clavate, apex obtuse, 4–4·5 × 2–2·5 μ; conidia hyaline, continuous, oblong-oval or narrow-oval, ends obtuse, 3–5 × 1·5–2·5 μ, or globose, 2–2·5 μ. On a spider, Vavuniya, Ceylon, December, 1923; ditto, Hakgala, Ceylon, May, 1912; ditto, Nuwara Eliya, Ceylon, January 1st, 1928 and July 15th, 1928.

The cultural details given in *Trans. Brit. Myc. Soc.*, *loc. cit.*, were derived from cultures from the specimen from Vavuniya.

Spicaria clavulifera Petch, n.sp. Purple-grey to ashy. Mycelium stout, 3–5 μ diameter, pale purple-brown. Conidiophores erect, crowded, covering the insect with a dense even pile, about 60 μ high, stout, 3 μ diameter below, expanding to 5 μ above, two or three-septate, terminating in a cylindrical head composed of an apical cluster of phialides, and usually a whorl of prophialides and phialides at the top of the penultimate segment, the phialides usually becoming parallel to one another; prophialides subcylindric, 8–12 × 3–5 μ; phialides cylindrico-clavate, 12–20 × 2·5–4 μ, narrowing above into a broad, conical, obtuse apex; conidia narrow-oval or oblong-oval, hyaline, continuous, ends obtuse, 5–7 × 1·5–2 μ. On a spider, Nuwara Eliya, Ceylon, October 3rd, 1926.

In culture on Quaker Oat agar, *Spicaria clavulifera* produced circular, white, rather compact patches, which developed purple-grey tufts of conidiophores from the centre outwards until each patch became a pulvinate mass. The type of growth was exactly similar to that of *Spicaria velutiformis*, and, as in the case of the latter, the agar was not coloured. Measurements of the phialides, etc., in culture were identical with those of the natural specimen. In culture, some of the old phialides had a stout cylindrical sterigma.

Spicaria clavulifera differs from *S. velutiformis* especially in the shape and size of its phialides. In general appearance the conidiophores of the two species are quite distinct, that of the latter having, in addition to the terminal, whorls of phialides some distance apart along the stalk, with divergent phialides, while on that of the former the cluster of phialides is apical, with at most one branch or whorl close below, and the phialides, though sometimes divergent, usually form a parallel bundle. Owing to the latter feature, the head somewhat resembles that of a *Penicillium*.

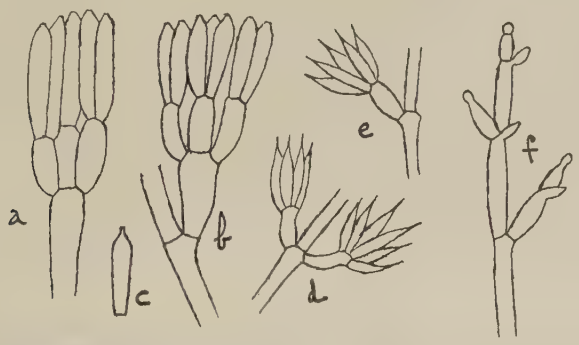

Fig. 5. *a*, *Spicaria clavulifera*, terminal cluster; *b*, *S. clavulifera*, lateral cluster; *c*, *S. clavulifera*, old phialide; *d*, *S. violacea*; *e*, *S. gracilis*; *f*, developing conidiophore of *S. gracilis*. × 1000.

Another coloured *Spicaria* was found on a Tineid case at Nuwara Eliya, forming a violet-grey pulverulent patch on the exterior. Its description is as follows.

Spicaria violacea Petch, n.sp. Mycelium purple-brown. Conidiophores clustered, up to 300 μ high, forming a violet-grey pulverulent patch; stalk septate, pale violet, 3–4 μ diameter, usually smooth, sometimes minutely rough; prophialides arising from the upper two to four nodes in whorls rather close together, sometimes the lowest whorl on short branches, the whole forming a globose head, about 40 μ diameter, or an ovoid head, about 60 μ high, 40 μ diameter; prophialides irregularly cylindric, expanded above, 6–8 × 3 μ; phialides flask-shaped, attenuated above somewhat abruptly, 8 × 2 μ; conidia globose, 1·5–2·5 μ diameter, or broadly oval, 2–2·5 × 1·5 μ, smooth, pale purple-grey, almost hyaline. On a Tineid case, Nuwara Eliya, Ceylon, December 3rd, 1927 (Fig. 5 *d*).

On Quaker Oat agar, growth extends over the whole slant, the culture being reddish purple and pulverulent. The reverse of the stroma is yellow, and the agar is coloured chrome-yellow at first, then orange-yellow to greenish yellow. Yellow elongated conical clavae are produced, at the margin of the slant or anywhere over

its surface; these ultimately become reddish purple, owing to the development of conidiophores on them.

Coremium gracile Petch was described in *Trans. Brit. Myc. Soc.* XI (1926), 260, from Ceylon specimens on spiders. The name is antedated by *Coremium gracile* Macbride in *Mycologia*, XVIII (1926), 125–31, a species from New Zealand.

In the original specimens of the Ceylon species the insect was enclosed in a silk web, the coremia developing on the web. A large number of specimens have since been collected at Nuwara Eliya, where it is one of the commonest entomogenous fungi, and these show that it is not confined to spiders, nor does it usually form coremia.

When the spider is enclosed in a web, the mycelium of the fungus emerges through the web and may form coremia, in addition to scattered or clustered conidiophores on repent strands. Conidiophores are also produced on the body or legs of the insect, inside the web. If the insect is situated between two leaves, fastened together by silken threads, the fungus produces conidiophores on the insect, and strands of mycelium which extend to the junction of the leaves and there give rise to coremia externally. But if the insect is merely attached to the leaf without any cover, the conidiophores are produced, singly or in clusters, anywhere on the insect or on strands of mycelium which spread from it over the leaf.

Coremium gracile Petch has also been found on lepidopterous larvae and pupae, on Tineid cases, and on *Lecanium* spp. On lepidopterous larvae it produces scattered conidiophores at first, and ultimately a continuous covering of mycelium and conidiophores. On pupae and Tineid cases it usually forms white pulverulent patches of crowded conidiophores. But if the pupa is embedded in wood or moss, one or more strands of mycelium emerge from the wood or moss and expand into a pulvinate tuft on the surface. Thus, on dissecting out the pupa in such cases, one obtains a more or less typical *Isaria*. On *Lecanium*, the fungus may form small coremia, or a loose fringe round the scale.

The identity of these various forms has been determined by culture. No coremia have been obtained in culture, the fungus in all cases producing an even pulverulent stroma.

In dealing with entomogenous fungi one soon learns that Hyphomycetes which normally are Mucedinaceae, *e.g. Beauveria Bassiana*, frequently produce coremia or Isarioid forms which would entitle them to be classed as Stilbaceae. Conversely, entomogenous species reckoned as Stilbaceae, *e.g. Isaria*, *Gibellula* and *Hirsutella*, usually produce, in addition to the compound form, conidiophores on the mycelium which covers the insect, and thus behave as normal Mucedinaceae.

The classification of such dimorphic species is, in general, governed by considerations of convenience. In the case of *Coremium gracile* Petch, the fungus is usually a Mucedine, the coremia or *Isaria* forms depending to a great extent on peculiarities of occurrence. As the ultimate conidiophore is a *Spicaria*, it would seem preferable to place the species in that genus, as *Spicaria gracilis* comb.nov.

It may be noted that the genus *Isaria* is a heterogeneous mixture of species which have little in common, except that they produce erect fascicles of conidiophores. As regards the entomogenous species, it is not possible to identify them, or to classify them in homogeneous groups, from their descriptions, because the actual conidiophore, *i.e.* the ultimate branch of the fascicle, has, in general, not been described. It is highly desirable that these species should be classified on the character of the conidiophore, *i.e.* not as *Isaria*, but as aggregate forms of *Oospora*, *Sporotrichum*, *Spicaria*, etc.

Coremium breve Petch in *Trans. Brit. Myc. Soc.* xi (1926), 259, on *Lecanium* sp., is *Spicaria gracilis*.

Spicaria gracilis Petch, comb.nov.; *Coremium gracile* Petch in *Trans. Brit. Myc. Soc.* xi (1926), 260; *Coremium breve* Petch, *op. cit.* p. 259. Conidiophores scattered, or forming white pulverulent tufts, or sometimes synnemata up to 1·5 mm. high, 0·4 mm. diameter below, with an irregularly ovoid head. Mycelium white, often forming repent strands. Conidiophores up to 120 μ high, 2·5–3 μ diameter, simple, or branched once at about half the height, or with two or three short branches forming the lowest whorl of the head. Head 20–50 μ high, of two to four whorls of prophialides and phialides and a terminal cluster. Prophialides cylindric, 5–6 × 2·5 μ; phialides distinctly flask-shaped, 6–9 μ high, 2·5 μ diameter below, attenuated from about half the height into a thin neck. Conidia in chains, hyaline, smooth, narrow-oval, ends acute, 1·5–3 × 1–1·5 μ, a few subglobose, 1–1·5 μ diameter. On a spider, Peradeniya, Ceylon, July, 1921; ditto, Hakgala, Ceylon, March, 1922. On *Lecanium* sp., Hakgala, Ceylon, March, 1922. On spiders, lepidopterous larvae and pupae, Tineid cases, and *Lecanium hemisphaericum*, Nuwara Eliya, Ceylon, 1926–28 (Fig. 5 *e*).

In culture on Quaker Oat agar, *Spicaria gracilis* forms an even pulverulent stroma, the conidiophores tending to be clustered in small tufts at first. The colour is white to cream, and the agar becomes yellow to orange-yellow in old cultures. On Dox agar, the reverse is orange-yellow, and the agar is not coloured.

Spicaria gracilis differs from *S. javanica* in the shape of its phialides, the size of the conidia, and the colour of the masses of conidia in culture, which in *S. javanica* is violet-grey.

A specimen on *Lecanium hemisphaericum*, collected at Nuwara Eliya, Ceylon, June 21st, 1927, is of interest. The fungus forms a narrow

white fringe round the scale, but is readily distinguished from *Cephalosporium Lecanii* by the fact that the hyphae, of which the fringe is composed, are more or less erect. The fringe in the only available specimen contains developing conidiophores, and the latter bear, at the upper septa, one or two developing phialides, or a prophialide with one or two immature phialides, or a branch with a cluster of phialides at the apex. The specimen was not at first recognised as *Spicaria gracilis*, but on taking it into culture it proved to be that species.

The interesting point is that these conidiophores and phialides (Fig. 5*f*) bear a close resemblance to the figure given by Johnston for *Spicaria Aleyrodis* (*Mem. Soc. Cubana Hist. Nat.* "*Felipe Poey*" III (1918), 79), and hence it would appear probable that the latter was a juvenile form of a species which may present a different appearance in a more mature state. It is not intended to suggest that *Spicaria Aleyrodis* is the same as *S. gracilis*.

Vuillemin (*Canadian Entom.* LVII (1925), 4, 97–9) has described another species of *Spicaria*, *S. canadensis*, on the larvae and pupae of *Stilpnotia salicis*. It is white, with pyriform phialides, 8–17 × 3 μ, and oblong conidia, 4–5·4 × 2·8–3 μ. This is evidently different from *Spicaria gracilis*.

46. *Acremonium Aranearum* Petch, n.sp.

This species is fairly common at Nuwara Eliya, Ceylon, on spiders, which it covers with a white, loose, more or less pulvinate mass. The mycelium bears phialides laterally, either at a node or scattered along the hyphae, sometimes only 2 μ apart. These phialides are elongated flask-shaped, or conoid, with an obtuse apex, or oval, with a fine short sterigma, solitary, or they may arise in pairs from an oval prophialide. Each phialide bears a single oval conidium, and after the abscission of the conidium, it collapses into a very fine thread, arising from the scar of attachment.

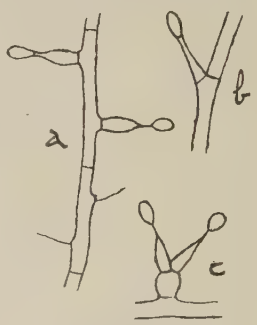

Fig. 6. *Acremonium Aranearum*. *a*, hypha bearing turgid and collapsed phialides; *b*, a phialide at a node; *c*, two phialides on a globose prophialide. × 1000.

A similar fungus occurs as a parasite on Mycetozoa in England. I have had specimens on *Comatricha nigra*, North Wootton, on *Cribraria* sp., Bawsey, and on *Arcyria cinerea*, Whitby. It has solitary oval conidia on flask-shaped phialides, which collapse into threads after maturity. I have not seen pairs of phialides on a prophialide in this species. I take this to be *Acremonium album* Preuss. It agrees with Preuss's figure, except that the latter shows simple sterigmata instead of phialides.

Rhinotrichum doliolum Pound & Clem., found on an undetermined Myxomycete in America, is, from the description, apparently the same as *Acremonium album* Preuss, though the describers give larger dimensions, stating that the hyphae are 5 μ in diameter. Their fungus is clearly not a *Rhinotrichum*.

These species differ from *Acremonium* in their evanescent phialides. That character does not appear to have been previously recorded. As it may be of common occurrence, and not peculiar to the species under discussion, they may be left in *Acremonium* until further evidence is available.

Acremonium Aranearum Petch, n.sp. Mycelium 1·5–2 μ diameter, regular, septate, covering the insect with a loose, white, pulvinate mass. Phialides lateral, at a node or scattered, elongated flask-shaped or conoid, 6–8 μ high, 1·5–2 μ diameter below, or oval, 2 × 1·5 μ, with a fine sterigma, 1 μ long; sometimes arising in pairs from an oval prophialide, 3 × 2 μ. Conidia hyaline, continuous, apical, solitary, oval, 2–5 × 1·5–3 μ. After abscission of the conidium, the phialide collapses into a fine thread. On spiders, Nuwara Eliya, Ceylon, March 29th, 1927, etc.

In culture on Quaker Oat agar, the growth is white and loose, and the agar is coloured purple-red. No plate-like spores, such as are recorded by Thaxter for *Desmidiospora*, have been found in nature, or in tube cultures and hanging drops.

47. *Rhinotrichum parvisporum* Petch, n.sp.

This species was first found in Ceylon on *Aspidiotus* and *Lecanium*. It occurred in a collection on leaves of *Hevea*, in which some of the *Aspidiotus* were attacked by *Pseudomicrocera Henningsii* and some of the *Lecanium* by *Aschersonia marginata*, but these fungi were not evident on the scales which bore the *Rhinotrichum*. The latter forms a white fringe round the scale, and spreads over it in a loose weft.

Fig. 7. *Rhinotrichum parvisporum*. Conidiophore. × 1000.

Another example of this species was found on an effete *Gibellula* on a spider.

The mycelium is 1·5 μ diameter, with lateral or terminal, simple conidiophores, occasionally in whorls or groups of three, up to 30 μ high. The conidiophores are 1·5 μ diameter, equal, attenuated above, or, more generally, 1·5 μ diameter below, becoming irregularly inflated up to 2 μ diameter, and thence attenuated and irregularly flexuose. In the specimen on *Aspidiotus*, etc., they bear fine sterigmata, each arising from a small conical base, total height up to 2 μ, over their whole length. In the example on a spider, the sterigmata are more crowded together, and almost all near the apex of the conidiophore, which is sometimes

acute and curved, sometimes slightly inflated. The conidia are borne singly on the sterigmata, and are hyaline, subglobose, 1–1·5 μ diameter, or broadly oval, 1 × 1·5 μ, shortly pedicellate.

In culture on Quaker Oat agar, the slant was covered by a continuous white felted stroma, pulverulent with conidiophores and conidia. The agar was not coloured. The conidiophores frequently occurred in groups on the mycelium, so that the fungus might have been taken for a *Cladobotryum* with decumbent conidiophores. The simple conidiophores in culture were up to 40 μ long, 2 μ diameter and slightly inflated below, tapering and becoming irregularly flexuose above, and bearing sterigmata on conical bases in the upper two-thirds.

Rhinotrichum parvisporum Petch, n.sp. Mycelium forming a white fringe round the insect and spreading over it in a loose weft. Conidiophores hyaline, simple, lateral or terminal, up to 30 μ high, 1·5 μ diameter below, equal, or irregularly inflated to 2 μ, and thence attenuated and irregularly flexuose; sterigmata linear, with a conical base, total height up to 2 μ, over the whole length of the conidiophore or near the apex; conidia hyaline, subglobose, 1–1·5 μ diameter, or broadly oval, 1 × 1·5 μ, shortly pedicellate. On *Aspidiotus* and *Lecanium*, attacked by other fungi (?), on *Hevea brasiliensis*, Dewalakande, Ceylon, January, 1928.

48. *Aspergillus depauperatus* Petch, n.sp.

This fungus occurred rather abundantly on *Lepidosaphes ulmi* on hawthorn at Hunstanton, Norfolk, in February, 1921, forming thin, white, byssoid patches extending partly over the insect and partly over the adjacent bark. As it was considered probably an abnormal state of some larger species, it was left unrecorded until a further investigation could be made. In 1928, it was collected at Nuwara Eliya, Ceylon, on an *Aspidiotus*, on which it formed minute white patches at the margin of the scale.

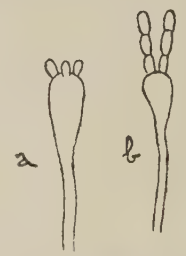

Fig. 8. *Aspergillus depauperatus*. *a*, conidiophore with developing conidia; *b*, *A. depauperatus*, with chains of conidia. × 1000.

The conidiophores are clustered, up to 50 μ high, 2–2·5 μ diameter below, stalk smooth, often irregularly curved, expanding gradually above into a pyriform or clavate head, 4–5 μ diameter, somewhat flattened above. Sometimes the conidiophore is inflated to 3·5 μ diameter below, contracting above, and then expanding into the head. The mycelium is septate, but the conidiophore is not. The head bears two to four chains of conidia directly, *i.e.* without any basidia or phialides. All the conidia fall away, leaving the empty head with two to four minute conical projections. The conidia are

oval, hyaline, smooth, 2–4 × 1·5–2·5 μ, with a few globose, 1·5–2 μ diameter.

In culture on Quaker Oat agar, the fungus forms minute white cushions, which coalesce into a continuous white crust. The fungus remains white, while the agar is coloured pink. The conidiophores in culture were not larger than in nature. The chains of conidia adhered laterally.

Though the head is small, the fungus appears to be an *Aspergillus*. It does not agree with *Spinalia* Vuill., *Dispira* v. Tiegh., or *Dimargaris* v. Tiegh. It has no sterigmata (phialides), and the lowest spore is the youngest, as in *Aspergillus*. *Briarea* Corda should differ in having a septate stalk, and in not having the apex of the stalk inflated.

Aspergillus depauperatus Petch, n.sp. White; conidiophores clustered, up to 50 μ high; stalk 2–2·5 μ diameter, smooth, not septate, often irregularly curved and inflated below, expanding above into a pyriform or clavate head, 4–5 μ diameter, flattened above; phialides none; chains of conidia two to four, sessile; conidia hyaline, oval, smooth, 2–4 × 1·5–2·5 μ, with a few globose, 1·5–2 μ diameter. On *Lepidosaphes ulmi*, Hunstanton, England, and on *Aspidiotus* sp., Nuwara Eliya, Ceylon.

THE LIFE-HISTORY OF *CYSTOCHYTRIUM RADICALE* OCCURRING IN THE ROOTS OF *VERONICA BECCABUNGA**

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(With Plate X and 19 Text-figures)

Introduction

Some years ago, while examining the roots of *Veronica Beccabunga* in order to collect material of *Ligniera Junci*, two other parasitic fungi were found closely associated in the cortical and epidermal tissues. Both were new species; the one, *Olpidium radicale*, was described some time ago by Dr E. J. Schwartz and myself(4), the other is the subject of this paper. Neither of these fungi is common, occurring, as far as is known, only in a single locality near Sevenoaks, Kent. Of the two *Olpidium radicale* is the more frequent, and seven years have elapsed before sufficient material of the second fungus, *Cystochytrium radicale*, could be accumulated, to work out the complete life history(3).

A remarkable fact which soon became apparent was the enormous number of individuals which occur in those roots which have become attacked, compared with the rarity of infected roots. Moreover all the individuals in a root are as a general rule in about the same stage of development. From this it was concluded that the power of the fungus to migrate from cell to cell of a root was far more efficient than its ability to spread from root to root even of the same plant. Very frequently only a single root of a plant was attacked, although in that root several hundred individuals might be present. As a result of this no pathological effect could be recognised in diseased plants whereby they could be distinguished from healthy ones.

Methods

Roots of *Veronica Beccabunga* were collected at frequent intervals during the whole of the year and examined for the presence of the fungus. When found, all the roots in the batch, whether diseased or not, were cut up and fixed in Bouin's fluid. Later they were examined separately and those showing infection were put aside; when a sufficient number had accumulated they were stained by the iron

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alum haematoxylin method using acid alcohol for differentiation. Records were kept of the time of year each collection was made in the hope of discovering a seasonal relation. Such a correlation, however, did not seem to exist, and the fungus could be found at all stages at any time of the year when the roots of the host plant were well developed.

Life-history

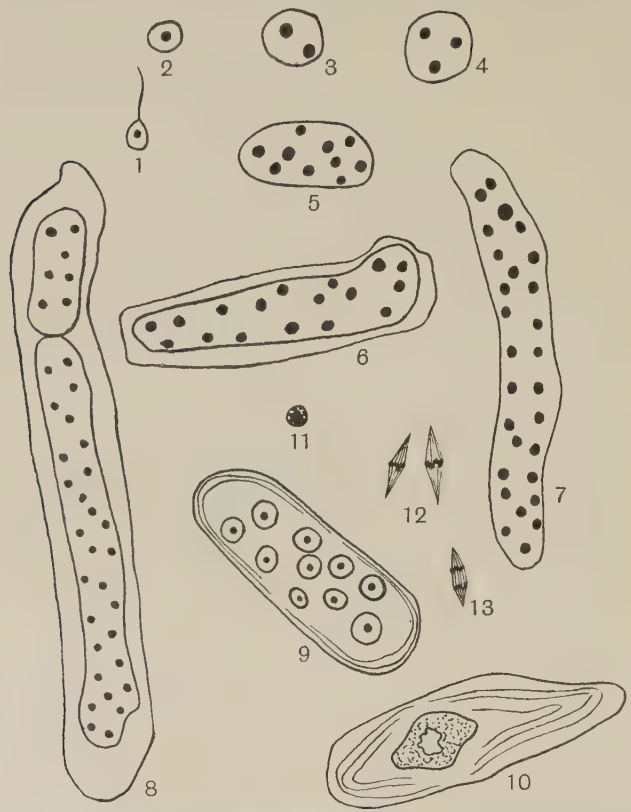
The earliest stage found was a small unicellular uninucleated body (Text-fig. 2) which consisted of a mass of densely staining peripheral cytoplasm with a small nucleus separated from the cytoplasm by a central vacuole. The nucleus was surrounded by a nuclear membrane and possessed a large central nucleolus (Text-fig. 11). At this stage the whole organism was surrounded by a thin membrane.

As growth continues the organism elongates and the nucleus divides mitotically. Increase in length is rapid and the fungus soon assumes an elongated oval shape (Text-figs. 3–9) whose length is ten to twenty times as long as broad. During this process the cytoplasm frequently becomes very vacuolated (Pl. X, figs. 3, 4) and in mature individuals a large number of nuclei are found embedded in cytoplasmic strands interspersed with large vacuoles. Scattered in the cytoplasm are numerous bodies which consist of chromatin. This vacuolate condition is not, however, always found, some individuals being completely filled with cytoplasm at this stage though in other respects identical with those already described. As the organism grows it frequently becomes swollen or curved until it acquires almost the appearance of branching (Text-figs. 14–19).

The cell wall now becomes thickened and a sheath composed of chitin is laid down around the organism. Frequently these sheaths are of considerable length, being as much as thirty times as long as broad. The cytoplasm at first fills the sheath, but later contracts away from the ends (Pl. X, fig. 2) and in some instances several masses are seen lying along the length of the cyst and are finally separated from one another by cross walls (Text-fig. 8). The result of this is the production of several zoosporangia lying in a row surrounded by a common membrane.

Each zoosporangium contains a large number of nuclei (Pl. X, fig. 5). The walls of the individual zoosporangia may become further thickened, thus rendering the study of the later development more difficult (Text-fig. 9). There is a reduction in the number of the nuclei, but there is no evidence of any fusion in pairs, and concurrently with this the cytoplasm is divided up. Later, by further separation and reduction in nuclei, a small mass of cytoplasm comes to surround only a single nucleus. From six to twelve zoospores are formed in each zoosporangium (Text-fig. 9). The mature zoospore

is spherical, possessing a single flagellum which is about twice as long as the oospore (Text-fig. 1). There is a single central nucleus

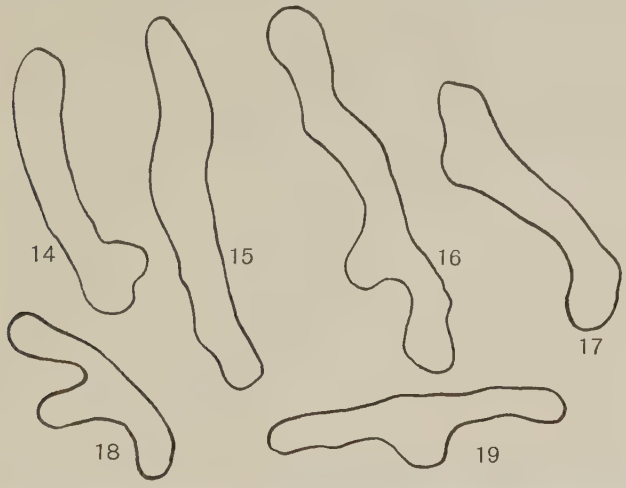


Text-figs. 1–13. Life-history of *Cystochytrium radicale*. 1. A zoospore with single apical flagellum. 2. Zoospore after entering the host tissue. 3. Binucleate organism. 4 and 5. Growth of the organism which is accompanied by multiplication of the nuclei. 6. An almost fully grown organism showing the chitinous sheath separated from the protoplast. 7. A fully grown organism in which the sheath has not separated from the protoplast. 8. A fully grown organism in which the protoplast has divided into two within the sheath. 9. The division of the protoplast into zoospores and the accompanying increase in thickness of the sheath. 10. The perforation of the wall of the sheath thus providing an exit for the zoospores, the drawing shows the enlarged area which has become thin as well as the smaller area which is actually perforated. 11. A nucleus in the resting condition. 12. The metaphase of the division; note the narrow elongated spindle. 13. The anaphase of the division. Figs. 1–10, ×1600; Figs. 11–13, ×2300.

and the cytoplasm is homogeneous. Oil globules are present, which, as the zoospore matures, tend to unite into a single large globule.

The wall of the zoosporangium now becomes perforated by a single aperture situated about the middle of its length, and through

this the zoospores migrate one at a time (Text-fig. 10). The perforation of the zoosporangium is apparently effected by pressure associated with a less extensive thickening of the wall at the point of rupture (Pl. X, fig. 6). The zoospores are relatively large, being about 12–15 μ in diameter. After they have emerged they swim about and then migrate into neighbouring cells of the host. Their further fate could not be followed in detail, but it appears that they have the power of penetrating the cell walls of the host and make their way either to the exterior or into other cells of the same root. It seems probable that the period of activity is brief and this may explain the preference which the zoospore shows for neighbouring cells rather than a new root for further development.



Text-figs. 14–19. A series of outline drawings showing the various shapes of zoosporangia found; in Figs. 16, 18 and 19 the protoplast was in process of division into two. The sheaths enclosing the protoplasts are not shown. All ×1500.

Cytological observations have not been very fruitful. Only a very few organisms have been found which showed clearly the type of nuclear division. It was clearly seen, however, that a typical spindle was formed and that there was a small but definite number of chromosomes arranged upon the equatorial plate during the metaphase (Text-fig. 12). All the nuclei of the individual were at the same stage in division (Text-fig. 13) and it is therefore concluded that the nuclei divide simultaneously. Owing to the small number of nuclei which could be examined, a count of the number of the chromosomes could not be made, nor would detailed considerations of the method of nuclear division be of value when based upon so small a number of observations. As far as could be made out, no centrosomes are present and no marked discharge of chromatin takes

place prior to nuclear division, as is characteristic of some of the Olpidiaceae and Synchytriaceae, though from the presence of chromatin granules in the cytoplasm, chromatin must be extruded in small quantities during the growth of the fungus. No evidence of any nuclear fusion was obtained nor has conjugation of the zoospores been observed. It seems likely in view of the close similarity in the size of the mature zoospore at the time of liberation and the first recognisable infection stage that no method of sexual reproduction occurs.

Discussion

The relationships of this new fungus are very obscure since it does not show any close resemblance to any of the other known types. In the opinion of several protozoologists it cannot be placed among the Protozoa, and therefore since it is devoid of chlorophyll or any other pigment it must be placed among the Fungi, and owing to its primitive nature in the Archimycetes (2). Of the groups of the Archimycetes to which it could be related two possibilities present themselves. Either it may be related to the Olpidiaceae, or the fungus belongs to the Hyphochytridiaceae. Whichever is the more probable there is no doubt that it must be included in the Chytridiales. The most important difference between these two families is the presence or absence of a mycelium. In the Hyphochytridiaceae a mycelium is present, but in the simpler forms it is only very rudimentary, consisting of little more than connections between zoosporangia; in the more complex types it may form a distinct rhizoidal structure. In the Olpidiaceae no mycelium is formed. Among the simpler members of the Hyphochytridiaceae such as *Hyphochytrium infestans* (6), there is very little differentiation into mycelium and zoosporangia, the organisms simply consisting of a series of zoosporangia formed by the swelling of an originally narrow tube which is looked upon as a short mycelium. In this respect it is similar to what is found in some of the cysts where a chain of several zoosporangia are formed. The zoospores in this family are uniflagellated as they are in *Cystochytrium radicale*.

On the other hand, the zoosporangia of *Cystochytrium* resemble those of the Olpidiaceae in some respects and the method of zoospore discharge is similar to what is found in *Olpidium radicale*. The shape of the cysts and the contraction of the contents to one end, which sometimes occurs, is found in *Olpidium radicicolum* (1) though generally as an abnormality. Chains of zoosporangia produced by constriction of a single cell are not found in the Olpidiaceae. The zoospores of *Cystochytrium radicale* are larger than those found in the Olpidiaceae.

The entire absence, as far as is known, of any sexual reproduction separates *Cystochytrium radicale* from both the Olpidiaceae and the

Ancylistaceae. There is, however, a certain morphological similarity between *Cystochytrium* and the recently described *Lagena radicicola* (5), which is found as a disease of wheat in Canada. Both the shape and development of the soma are similar and the same vacuolation occurs in both organisms, both are multinucleated and in a general way the vegetative stages seem remarkably alike. In *Lagena*, however, there is a definite exit tube and the zoospores are biflagellated. Moreover, sexual reproduction of an advanced type has been described which at once places this fungus in the Ancylistaceae.

As far as I am aware, no other member of the Chytridiales resembles *Cystochytrium radicale*, and its true systematic position must await the description of further species; there seems little doubt that many more chytridiaceous fungi will be discovered when a more extensive examination of roots of plants, not necessarily showing any pathological effect, is made.

In the present state of our knowledge it seems most reasonable to include *Cystochytrium radicale* in the Hyphochytridiaceae as a new genus somewhat closely related to *Hyphochytrium*. A preliminary account of it under the name *Cystochytrium radicale* has already been published (3).

Diagnosis

Cystochytrium *gen.nov.*

Soma uninucleatum fit multinucleatum et in vagina chitinosa includitur. Corpus maturatum in zoosporangium conversum paucas zoosporas uniflagellatas parit.

C. radicale *sp.nov.*

Corpus uninucleatum, nucleis mitotice se dividentibus, fit multinucleatum. Soma maturatum in vagina chitinosa, quae saepe maior est quam corpus ipsum, includitur. Vagina crassiore facta zoosporangium formatur et in circiter sex zoosporas dividitur. Zoosporae per fistulam in vagina effugiunt. Zoosporae sunt globosae 12–15 μ diam. et uniflagellatae; aut in alios radices aut in alias eiusdem radicis cellas effugiunt.

Hab. In cellulis radicum Veronicae Beccabungae ad Sevenoaks, Kent in Britannia.

Summary

1. A new species of fungus belonging to the Chytridiales is described under the name *Cystochytrium radicale*, which attacks the roots of *Veronica Beccabunga*.

2. A uninucleated zoospore enters and grows within the cells of the host, later becoming multinucleate and enclosed in a chitinous sheath. When mature the soma functions as a zoosporangium and

produces about eight zoospores which escape through a pore in the thickened wall of the zoosporangium.

3. The zoospores are spherical, 12–15 μ in diameter, and possess one flagellum. They make their way out of the root and swim in the water around them and infect fresh roots.

4. The host plant is not visibly affected by the presence of the fungus which sometimes occurs in a particular root in large numbers.

5. The relationships of the fungus are discussed, and it is suggested that it should be placed in the Hyphochytridiaceae, in a new genus somewhat closely allied to *Hyphochytrium*.

I wish to thank Dr E. J. Schwartz for his assistance in making some of the collections at Dunton Green and also for his help in examining the slides.

EXPLANATION OF PLATE X

The photomicrographs were made with a Zeiss Seidentopf photographic eyepiece at tube length of 115 mm., using a Zeiss 2 mm. (N.A. 1·4) objective (Figs. 3–6) and a Zeiss 4·2 mm. (N.A. 0·65) objective (Figs. 1–2).

Fig. 1. General view of the organism lying in the cells, showing the host nucleus lying along the side of the parasite. ×250.

Fig. 2. Some mature zoosporangia which have contracted away from the sheath which can be seen in the lower part of the fourth zoosporangium from the left. ×250.

Fig. 3. A young zoosporangium showing an early stage in the vacuolation of the cytoplasm, also the host nucleus associated with the fungus. ×550.

Fig. 4. A later stage in the vacuolation of the cytoplasm showing the chromatin lying in the cytoplasm of the organism. ×550.

Fig. 5. A large zoosporangium slightly vacuolated just prior to zoospore formation. The nuclei are shown stained dark. ×550.

Fig. 6. A photograph taken of living material showing the mature zoosporangium after the zoospores have commenced to escape and two zoospores which had just emerged prior to taking the photograph. The apical end of the one on the left is directed upwards and of the lower one towards the left. The nucleus of the zoospores is clearly seen though no stain had been applied to the material. ×550.

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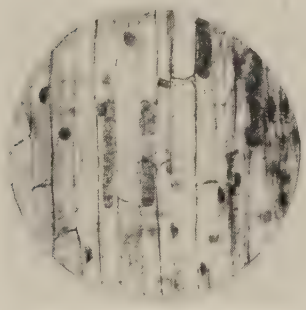

Fig. 1

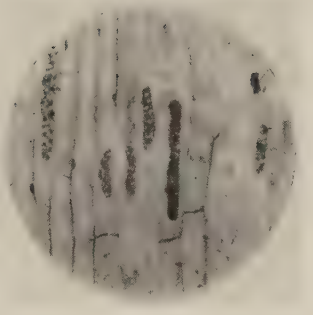

Fig. 2

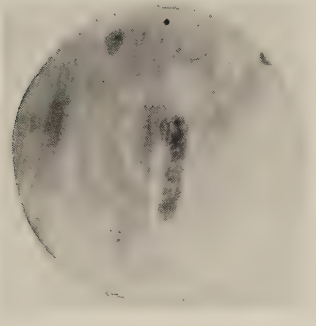

Fig. 3

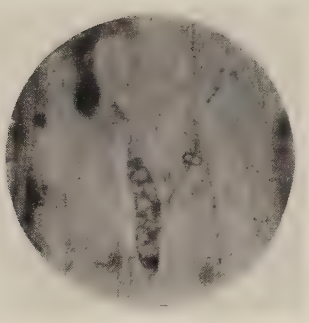

Fig. 4

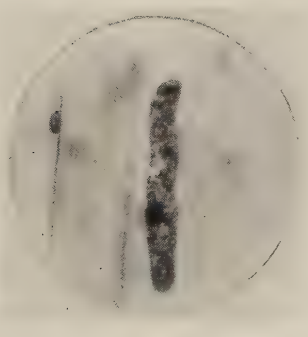

Fig. 5

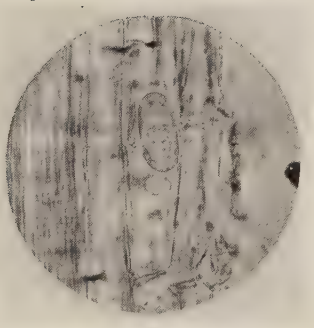

Fig. 6

Cystochytrium radicale Cook

OBSERVATIONS ON FOOT-ROT DISEASES OF CEREALS

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(With Plate XI)

Introduction

In the past little attention has been given in England to the incidence of those diseases affecting the base of cereal plants, to which the name of foot-rots has been given. Their widespread occurrence and destructive nature are just beginning to be recognised in this country although little is as yet known regarding the causal organisms and the environmental conditions affecting their attack. In the Eastern Counties it is exceptional to find a field of wheat at the time of earing which on inspection fails to reveal cases of foot-rot; though in many fields the damage caused in normal seasons is too slight to call for any action on the part of the farmer, in others this is by no means the case. Engledow and his co-workers (8), in their statistical investigation of the yield of wheat, record a loss due to this group of diseases amounting to 30 per cent. of plants in the latter half of crop life in a field near Cambridge; and, while I have made no statistical observations on their incidence, I am convinced that in some of the fields which were examined this figure must have been exceeded.

The symptoms of foot-rot call for little mention here since they have been fully described by Bennett (3) in this country, Davis (7) in America, and various writers in other countries. Although the disease may exhibit itself at any stage of the plant's growth from the time of germination, when a seedling blight may be caused, up till the time of maturity, it is most obvious in the six weeks preceding harvest during which the ears are filling, turning yellow, and ripening. It is then that it can commonly be seen in the form referred to as white-heads and thinning-out, involving a bleaching of the ears which fail to produce sound grain, and a browning at the base of the stems which frequently lean over instead of standing stiffly erect as healthy stems do. Occasionally associated with these symptoms is the condition known as ear-blight in which the fungus, instead of being confined to the base of the plant, is present in the ear itself, thus causing both loss in yield and infection of the grain which may be used as seed the following year.

The investigation which is here described was essentially pre-

liminary in character, its main object being to gain some idea of the prevalence and importance of foot-rot around Cambridge and to assess the part played by parasitic fungi in its occurrence. It was felt that until more was known about the cause or causes of the disease, little could be done on the greater problem of its dependence on weather and soil factors and its response to measures of control.

Previous work in England and in other countries

In 1884, W. G. Smith described a "straw-blight" of wheat, barley and rye consisting of attack of the stem at soil level by a fungus, which, while usually confined to the basal internode, occasionally extended to the second or third. He recorded that the loss from this disease ranged up to half of the crop, being greatest in long-continued warm moist weather. Diseased stems were browned at the base and were seen to contain hyaline fungal hyphae, but no spore form was recorded and the fungus was not named.

In 1912, the Board of Agriculture and Fisheries published a leaflet on "take-all" (6), the foot-rot caused by *Ophiobolus graminis*; it was stated that this fungus had been found attacking wheat in England, and that it had been isolated at Kew from oats sent from North Wales. It was suggested that the disease was far more prevalent in this country than was generally suspected and that the fungus was very apt to be overlooked, since, during the period of growth of the cereal only the mycelium was present, fruiting bodies being produced on the stubble during the winter months. Possibly as a result of this statement it has sometimes been assumed without further proof that fields showing the symptoms of whiteheads and deafness of ear were suffering from attack by *Ophiobolus*. Careful identifications have, however, been made in sufficient number to indicate that this fungus occurs in various parts of England, having been recorded from the Northern Counties by Bennett (3), from the West Midlands by Preston (13) and from East Anglia by Biffen (5). Jones (11) mentions the occurrence in Wales of perithecia in large numbers early in August on oats affected with take-all.

In the year 1923 F. T. Bennett began investigations of the disease known as thinning-out, whiteheads, and deaf-ears, which had been the cause of considerable reduction in yield of wheat on a farm in Yorkshire during the preceding ten years, and which he subsequently found widespread in Northumberland, Durham, Cumberland and Yorkshire. He proved that *Cladosporium herbarum*, which so often appeared as a black growth on diseased ears, was not the primary cause of the trouble, for he was unable to infect healthy plants with this fungus at any stage of their growth (2). He collected diseased material from four farms some distance apart and made fungal isolations from the bases of the stems. He found that while some plants

were attacked by *Ophiobolus graminis*, others were free from this fungus but showed species of *Fusarium* at their base; the two genera were almost equally common (3). The material affected with *Fusarium* yielded two species, *F. culmorum* and *F. avenaceum*; both were shown to cause a foot-rot of wheat, oats, barley and rye involving a blight of the seedlings, a rotting of the crown and roots of such plants as survived, and finally the production of whiteheads and deaf-ears, an effect similar to that seen under natural conditions in the field. Inoculation of the ripening ears of wheat with conidial suspensions of these two fungi led to the incidence of a severe epidemic of ear-blight. Further he showed that they were amongst the chief causes of foot-rot in northern England. He has shown more recently (4) that *Gibberella Saubinetii* occurs on cereals in this country, having isolated it from grains of wheat undergoing germination tests at the National Institute of Agricultural Botany, Cambridge, from barley seed in Durham, and from the bases of wheat and barley plants grown in the Eastern Counties.

Helminthosporium sativum has once been recorded as a cause of foot-rot in England, N. Smith (14) having isolated it from specimens of affected barley near Cambridge.

Foot-rot diseases appear to occur wherever cereals are grown. In the United States and Canada considerable losses are caused by these diseases, the most destructive organism being *Helminthosporium sativum*, with *Gibberella Saubinetii*, species of *Fusarium*, and *Ophiobolus graminis* not far behind. In Australia much loss is caused by *Ophiobolus*, but Hamblin (10) thinks that in some seasons *Helminthosporium sativum* does more damage than the true take-all. *Wojnowicia graminis* is also found associated with these fungi. In Europe, on the other hand, *H. sativum* is of little importance and most of the foot-rot damage is stated to be caused by species of *Ophiobolus* and *Leptosphaeria*, and by *Calonectria graminicola*. Foëx and Rosella (9) attribute most of the damage caused by foot-rot in France to *Ophiobolus graminis* and to an unidentified fungus which they picturesquely designate as "le champignon des taches ocellées." They suggest that *Ophiobolus herpotrichus*, *Leptosphaeria herpotrichoides*, and *Wojnowicia graminis* are only weakly parasitic and cause little harm in the field.

Methods

As the investigation had the twofold object of obtaining some idea of the distribution of the foot-rot diseases in the neighbourhood of Cambridge, and of finding out what parasitic fungi might be associated with the foot-rot symptoms, it called for observations on field crops as well as laboratory work on diseased specimens Field inspections were started early in July, 1929, when the wheat was for the most part in the ear though still green, and the whiteheads stage of

the disease was most readily to be seen. Observations were continued till late in August, when most of the fields had been harvested. They were resumed when, in March of the following year, the effect known as Spring Yellows became apparent in fields of autumn-sown cereals, and were continued till about the middle of August. While particular attention was given to the disease as it occurred on wheat, its incidence on oats and barley, and to a less extent on rye, was noted. Cereal fields were inspected over an area extending from Hatfield in Hertfordshire on the one side to near Mildenhall, Suffolk, on the other, most of the observations being made, however, within about six miles of Cambridge. In each field a rough estimate of the amount of disease present, whether as seedling-blight, whiteheads or ear-blight, was made and affected plants were taken to the laboratory and examined for signs of fungal attack on stems and ears. Where this was suspected, portions of the plant were cut off and sterilised externally by a method recommended by Bennett. The affected portions were placed in a stoppered flask of water which had been boiled to remove all air. They were left to soak for two or three hours with occasional stirring and were then removed, plunged into 25 per cent. alcohol for one minute, into 1 per cent. formalin for three minutes and washed in sterilised water. Finally they were transferred to sterile test-tubes containing a wad of damp cotton-wool at the base and left at room temperature for a few weeks. Usually a strong fungous growth was produced on the specimens, indicating that a fungus had been present within the stem. The cultures were examined, where possible the fungus was identified, and a number of pure cultures were started from single spores.

Occurrence of seedling-blight

During the latter half of April and throughout May it was common to see fields of spring-sown barley with areas of yellow in place of the normal green. Sometimes the whole field showed this discoloration, while in others there were sharply localised patches, usually in parts of the field where drainage was bad and particularly where water had lain on the land. Severely affected plants showed yellowing of all the leaves, but in less severe cases yellowing was confined either to two or three outer leaves, the inner ones remaining green, or to one or two inner leaves, the outer being green. Most of the yellowed plants examined contained within the leaf-sheaths at the base of the stem the whitish grub of the wheat bulb fly, *Hylemyia coarctata* Fall. In many, however, the yellowing was found to result from infection of the young leaves by *Helminthosporium gramineum*, while in some it appeared to be the effect of wireworm attack. Only a dozen plants were obtained in which the discoloration could not be attributed to one of these three causes. Portions from the base

of the shoot were taken from these plants, sterilised externally and incubated. The organisms obtained from them are shown in Table I.

Table I. *Showing the organisms obtained from twelve yellowed seedlings of barley collected from various fields in the spring of* 1930.

Organism	No. of plants affected
Fusarium culmorum	2
F. culmorum, Helminthosporium sativum	1
Unidentified *Fusarium* spp.	4
Mucor, bacteria, etc.	2
Nil	3

Thus species of *Fusarium* were isolated from seven seedlings and *F. culmorum* from three of these, of which one was also infected with *Helminthosporium sativum.*

Fields of wheat, unlike those of barley, showed very little of the spring yellows symptom and in the few places where it occurred it was usually associated with attack by wheat bulb-fly or wireworm No fungal organisms were isolated which might have produced this effect.

During May a few fields of oats were seen with conspicuous yellowing of the young plants. Generally the base of the shoot had been gnawed, and the soil around affected plants contained wireworms. One field was seen in which considerable browning of the seedlings was caused by *Helminthosporium Avenae.*

In short, the observations made on the incidence of seedling-blight during the spring of 1930 suggested that foot-rotting fungi were playing an insignificant part in its causation, and that of far greater importance were such insects as the wheat bulb-fly and the wireworm, and the leaf-infecting fungi *Helminthosporium gramineum* and *H. Avenae.* The greater severity of attack on poorly drained portions of land is possibly due to the lowered vitality and consequent reduced power of resistance of plants growing under unfavourable conditions.

Occurrence of whiteheads and thinning-out

Foot-rot diseases manifested by the symptoms of whiteheads and thinning-out were found to be extremely prevalent on all cereals during the summer of 1929. Of more than sixty fields examined, almost all contained some plants showing these symptoms. In some of the fields infected plants were so infrequent as to necessitate a very close search before any could be found, but in others much of the crop was found to be suffering from disease. Most of the fields showed an intermediate degree of infection with foot-rotted specimens easily to be seen but occupying no very considerable proportion of the crop. A number of affected stems on being cut open were found to have a dense mycelial growth occupying the hollow straw in the

region of the basal internode, and frequently pinkish crusts of conidia of *Fusarium* were to be seen on the basal nodes and on the withered leaves sheathing the base of the stem. Often, however, a superficial examination failed to reveal any signs of the fungus on diseased stems and it was observable only after the treatment which has been described. The fungal organisms which were isolated from the basal portions of 163 infected plants, representing 58 different fields, are shown in Table II.

During the summer of 1930, whiteheads and thinning-out proved to be fully as prevalent as during the preceding summer, and they were found in almost every field of cereals which was inspected. Fungi were obtained from portions of 212 affected plants collected from 72 different fields; the genera concerned are shown in Table III.

Table II. *Showing the fungi isolated from* 172 *foot-rotted stems in* 1929.

Organisms isolated	Number of plants affected			
	Wheat	Oats	Barley	Rye
Fusarium culmorum	47	25	29	4
Fusarium spp. unidentified (micro-conidia only)	5			
Fusarium spp. unidentified, with slender spores	5	1		
Helminthosporium sativum	2			
Alternaria, Cladosporium, etc.	18	2	1	2
Sterile mycelia unidentified	31			
Total number of plants examined	100	28	30	5

Table III. *Showing the fungi isolated from* 212 *foot-rotted stems in* 1930.

Organisms isolated	Number of plants affected		
	Wheat	Oats	Barley
Fusarium culmorum alone	33	9	25
„ „ with other fungi	11	1	7
Fusarium spp. unidentified (micro-conidia only)	15	1	6
Alternaria, Cladosporium, etc.	16	1	1
Fungi not identified	65	8	17
Total number of plants examined	137	20	55

The figures given in Tables II and III indicate the presence of *Fusarium culmorum* in a high proportion of foot-rotted plants. From Table II it will be seen that, of 163 affected cereal stems, 105 or 64 per cent. yielded this fungus. In wheat alone the proportion is lower, working out at 43 per cent., but for the remaining three cereals taken together it reaches the high figure of 92 per cent. Of the 212 affected stems recorded in Table III, 86 or 41 per cent. contained this species. Its widespread distribution is further revealed by a consideration of the percentage of fields inspected in which it occurred, the data being recorded in Table IV.

The figures given in Table IV show that *Fusarium culmorum* was found in a high proportion of the fields examined, and it seems

probable that this fungus occurs throughout the cereal-growing land in the Cambridge district.

Table IV. *Showing the number of fields inspected in which* Fusarium culmorum *occurred.*

Cereal	No. of fields inspected 1929	No. with *F. culmorum*	No. of fields inspected 1930	No. with *F. culmorum*
Wheat	37	27	45	28
Oats	10	8	11	7
Barley	9	9	16	13
Rye	2	2		
Total	58	46	72	48

As is evident from Tables II and III, many of the fungi isolated from foot-rotted specimens were not identified, mainly through lack of time. They included several cultures of *Fusarium* which, owing to bacterial contamination or other causes, failed to produce spore-forms other than micro-conidia which proved of little use as a means of identification. It is probable that some, if not all of these cultures, should go to swell the numbers of *F. culmorum*, since the vegetative characters were not dissimilar. Two species of *Fusarium* were isolated which possessed slender micro-conidia unlike those of *F. culmorum*, and which differed from each other in cultural characters. They have not been identified specifically, and will be referred to as W 1 and W 10.

Two strains of *Helminthosporium sativum* were isolated from stems of wheat; this apparently constituted the first recorded occurrence in this country of this species on wheat affected with foot-rot. Each strain was found only once and the two plants concerned were obtained from widely separated sources. It will be noted that *Ophiobolus graminis*, generally believed to be the main cause of white-heads, is not recorded in either Table II or Table III. I am of the opinion that this fungus may be among the organisms isolated but that, because it failed to produce perithecia, it was classed with sterile mycelia. A number of these were cultured on various artificial media as well as on the cereal straw, but perithecia were never seen. That this is not conclusive evidence of the absence of *Ophiobolus* is shown by Davis (7) who found that, of the three strains of this fungus he investigated, two failed to form mature perithecia under any of the conditions which he tried, and the third produced them only intermittently. It is of interest that Foëx and Rosella (9) obtained from foot-rotted plants an actively parasitic fungus which failed to produce any fruit-bodies. Their description of the symptoms produced by this fungus and of its behaviour in culture suggests that this fungus was amongst the sterile mycelia recorded in Tables II and III.

The pathogenicity of certain of the isolated fungi towards cereals in the seedling stage was tested in a series of experiments, the results of which will be given in a succeeding section.

Occurrence of ear-blight

In the course of the field inspections during the summer months, a close look-out was kept for cases of ear-blight, a form of the disease which is frequently found associated with the foot-rots due to *Fusarium* species in other countries, especially in North America. In spite of the great amount of foot-rotting which occurred during 1929, blighted ears were found but rarely. This was perhaps to be expected since the season up till August was very dry and, to quote Atanasoff(1), "dry weather with slight winds during and after the period of blossoming will practically eliminate blight infection although all the other necessary conditions may be present."

Portions of ears thought to be blighted were removed, sterilised by the formalin method used for stems, and incubated in sterilised test-tubes. A number of these yielded only saprophytic organisms including species of *Penicillium*, *Cladosporium*, and *Alternaria*, but undoubted cases of Fusarial head-blight were found on three out of the four cereals.

Although the ears of over two hundred stems showing the whiteheads symptoms were examined, only eight were found to be infected with *Fusarium*; four of these were wheat, three barley, one oats, none of rye. The eight ears affected with head-blight came from different fields; in no field was more than a single instance of head-blight noticed. *Fusarium culmorum* was always responsible for the blight. In this connection Atanasoff(1) made the interesting statement that, while this species seldom causes blighting of ears in the wheat-growing region of the United States, it is the common cause of head-blights of the cereal crops in Holland.

The summer of 1930 was at first unusually dry, and it was only towards the end of July and onwards throughout harvest time that heavy rain occurred. Ear-blight was little more common on wheat than in the previous year, being found in only seven fields and only in isolated specimens; it was not seen at all on barley and oats. In estimating its occurrence in the field careful examination was necessary, since attack by the small orange-coloured larvae of the wheat midge, *Cecidomyia tritici*, abundant in 1930, simulated closely the blight due to fungus. In every instance the fungus was identified as *Fusarium culmorum*.

Although blighting of the ears was of negligible importance in the Cambridge district during the dry summer of 1929 and the following summer, the first half of which was dry, it is possible that in a favourable season this symptom might assume more serious proportions,

since the causal fungus is present in sufficient quantity as shown by the widespread occurrence of whiteheads due to it.

Pathogenicity tests of fungi isolated

A number of tests were made with some of the fungi isolated to determine which are of importance in causing the disease. Those tested include the three species of *Fusarium* and the two strains of *Helminthosporium sativum* obtained from wheat. Their pathogenicity towards cereals in the seedling stage growing under greenhouse conditions was tried, the method adopted being as follows:

Grains of the cereal were disinfected externally before sowing. They were soaked over-night in water and then placed for five minutes in a 0·5 per cent. solution of mercuric chloride in 50 per cent. alcohol, and kept well stirred. Thereafter they were washed in sterile water or in tap water. The soil used for most of the tests was not sterilised, for it was thought that, consisting as it did of potting soil brought from the Botanic Gardens, it should be free from organisms likely to cause foot-rot of cereals. It was placed in 7 in. pots and twelve grains were sown in each pot. The grain was dipped just before sowing in a concentrated spore suspension of the fungus, and, as a rule, this suspension was afterwards used to water the pots. In each test some uninoculated grain was sown to serve as a control. Inoculated and control pots were left in a greenhouse, heated during the winter months, where they were kept under observation.

Fusarium culmorum, *cultures W* 3 *and W* 4

A preliminary test indicated that the two cultures of *F. culmorum* were pathogenic to wheat in the seedling stage. Inoculated grain was sown in sterilised soil and two or three seedlings were uprooted and examined each day starting from the second day. By this means it was possible to observe and follow the course of the destructive seedling blight which resulted.

Subsequently culture W 4, an isolation made from wheat, was tested on seedlings of wheat, oats and barley. These were grown in pots containing inoculated soil for twelve weeks, and then uprooted. Although inoculated plants differed little in size from the uninoculated controls, they nevertheless showed signs of attack in the form of browning at the base of the stem, faint in barley, more obvious in wheat and very marked in oats, where pinkish crusts of conidia of *Fusarium* were to be seen on the basal leaf-sheaths. From affected plants of all three cereals, *Fusarium culmorum* was re-isolated. This experiment shows that a fungus derived from one cereal may be equally virulent to another cereal.

A small plot of wheat, half of which was inoculated at the time of sowing with *F. culmorum*, was laid down at the Botanic Gardens in

order that the course of the disease might be followed under less abnormal conditions than those prevailing in pot experiments. Unfortunately most of the stems were broken by sparrow attack and the symptom of whiteheads was not seen. Foot-rotting was much less severe in this plot than in the adjacent one inoculated with *Helminthosporium sativum*, but *Fusarium culmorum* was re-isolated from the base of two out of three plants tested.

Fusarium *sp.*, *cultures W* 10 *and O* 3

The conidia of this species were found on several occasions forming pinkish masses on the basal nodes and withered leaves of diseased plants, but although three or four tests were carried out, no signs of infection appeared on wheat seedlings under greenhouse conditions. In the field it probably occurs merely as a secondary organism in stems already rotted by an active parasite. In view of its lack of virulence, no attempt was made to identify the species.

Fusarium *sp.*, *culture W* 1

This species was similar in possessing spores of long, slender type and in failing to prove in the slightest degree pathogenic under greenhouse conditions. It is probably of no significance as a cause of foot-rot in the field.

Helminthosporium sativum, *culture W* 5

In a preliminary test Little Joss wheat was sown in two pots one of which was inoculated with spores of culture W 5. When the seedlings were a few weeks old, several plants were removed and the basal portion of the stem was disinfected externally with formalin, washed in sterilised water, and incubated in a sterile test-tube, in order to see if any infection by the fungus had taken place.

Half of the plants from inoculated pots and none from the controls yielded the fungus, suggesting that this strain is definitely pathogenic, a conclusion that has been amply confirmed in subsequent tests.

In a more critical experiment, the effect of the fungus on the three cereals, wheat (Little Joss), oats (Abundance), and barley (Spratt Archer) was tried. Three pots of each cereal were sown, two being inoculated while the third was left uninoculated to serve as a control. The pots were placed in a heated greenhouse and kept well watered. The germination of none of the cereals was affected significantly by the presence of the fungus. On the eighteenth day two or three of the seedlings of wheat were seen to be distinctly dwarfed and yellowed. One was removed and the base of the stem was found to be browned and rotted. The coleoptile and base of the shoot were found to be full of fungal hyphae. Nine weeks after sowing a striking difference was apparent between the wheat plants in

inoculated pots and those in the control. Several of the former had been completely killed and the surviving plants were weak and stunted with their outer leaves yellowed and withered; the control plants were stout and healthy. The contrast in appearance is shown in Pl. XI, Fig. 1, a photograph taken about nine weeks after sowing. In barley and in oats, no appreciable difference was visible between the plants in inoculated and in control pots up to sixteen weeks from the date of sowing. By that time, of the twenty wheat plants contained in the two inoculated pots all but two had been killed off. One of these had an abnormal dwarfed tufted appearance due to the presence of no less than twenty-six tillers, and was shown to be infected with the fungus. Both barley and oats produced ears and, nineteen weeks after sowing, the whiteheads symptom was seen rather indistinctly in the pots of inoculated barley. Some of the stems were badly browned at the base and of five tested, two yielded *H. sativum.* Barley plants from the control pot failed to yield this fungus nor was it obtained from any plants of oats. In this experiment, therefore, while inoculated wheat plants were destroyed by the fungus, barley showed only slight infection and oats none at all.

In November, a plot of wheat was sown at the Botanic Gardens, the grain for half of which was dipped in a culture of *Helminthosporium sativum*, W 5, just before sowing. By June, pronounced signs of attack were to be seen in the inoculated half of the plot. A number of plants were killed off and there were numerous gaps in the rows. In many plants the stems were prostrate, lying along the ground in all directions and badly browned at the base where they broke off easily, owing to thorough rotting of the crowns. On such plants a black velvety layer of conidiophores covered the base of the stem, and spores were also found on ears and leaves. The fungus was isolated without difficulty from portions of stem-bases sterilised externally. In the control half of the plot the plants remained healthy and no sign of the fungus was to be seen, the contrast between the two halves of the plots being most marked.

Helminthosporium sativum, *culture W* 17

Less extensive tests were carried out on culture W 17, but they were sufficient to indicate that the pathogenicity of the two strains was similar. Pots were sown with wheat, oats, and barley, and in some the grain was inoculated with the fungus while in others it was left uninoculated. Three weeks after sowing, the wheat seedlings in the inoculated pots were looking unhealthy, some being slightly discoloured and all of different sizes. The wheat seedlings in the control pots and all the barley and oat plants appeared healthy and vigorous. After ten weeks severe attack was obvious on the inoculated wheat; several plants were dead and others were badly browned at the base.

From such plants the fungus was readily re-isolated. The inoculated barley showed a much less degree of attack, but some plants were slightly browned at the base and yielded *H. sativum*. On oats there was no obvious browning though one stem yielded the fungus after surface sterilisation. In this second strain, therefore, as in the first, attack is much more severe on wheat than on barley and is of the slightest on oats under the conditions of the experiment.

Strains of *Helminthosporium sativum* causing foot-rot

Special consideration was given to the two strains of *Helminthosporium sativum*, because this species had not previously been recorded as causing foot-rotting of wheat in this country, though recognised in America and Australia as one of the chief causal organisms. Since it appeared on only two out of one hundred wheat plants suffering from foot-rot, which were examined in the first year, and in the following year it failed to appear at all on wheat, this fungus cannot be regarded as occurring yet in any abundance in the district or as being of any economic importance. At the same time, the extreme destructiveness of one of the strains on a plot of wheat in the Botanic Gardens as well as in the greenhouse, in both of which situations it proved much more virulent than *Fusarium culmorum*, suggests that if the fungus were to spread and secure a firm hold in the district it might cause considerable damage in certain seasons. The two strains referred to as W 5 and W 17 were obtained from the base of two wheat stems, the one collected from Bunker's Field, University Farm, the other from a field near the village of Wicken, approximately twelve miles distant. In both fields foot-rot was very common but appeared to be due mainly to *Fusarium culmorum*.

The two strains when grown on potato dextrose agar exhibit significant differences in their types of growth, as can be seen from Pl. XI, Fig. 3. Microscopic examination, however, fails to reveal any obvious feature by which the two strains might be differentiated. In both the spores are large, dark olive-brown, and slightly curved, and as a rule there are several on each sporophore, borne at intervals along its length. The sporophores are unbranched, geniculate and brown. The spore dimensions of the two strains when growing under similar conditions were compared. For the purpose, 2 per cent. potato dextrose agar was made up and an equal volume poured into each of several plates, which were then inoculated and left in an incubator at 26° C. After three weeks, two hundred spores of each strain were measured. The results obtained are given in Tables V and VI, which also give the corresponding figures for conidia produced in tubes containing moist fragmented ears of wheat, and for the conidia of one of the strains produced, under natural conditions at the base of infected wheat plants in the plot at the Botanic Gardens.

Table V. *Showing the spore dimensions of two strains of* Helminthosporium sativum (*measurements in* μ)

Culture	No. of spores measured	Length				Breadth			
		Mean	Standard deviation	Max.	Min.	Mean	Standard deviation	Max.	Min.
W 5, potato dextrose agar for 3 weeks	201	33·7	14·7	77	12	15·5	3·15	25	10
W 17, ditto	200	31·0	12·1	66	12	15·3	2·7	23	10
W 5, moist wheat ears for 5 weeks	100	51·4	11·6	69	19	17·9	2·8	23	11
W 17, ditto	100	51·1	13·7	73	19	19·9	2·9	31	11
W 5, on infected wheat in the field	201	84·4	13·8	116	32	20·5	2·5	27	11½

Table VI. *Giving the results of septa counts of spores of the two strains*

Culture	No. of spores measured	Mean septa no.	Standard deviation	Max. no.	Min. no.
W 5, potato dextrose 3 weeks	327	3·4	1·7	7	0
W 17, ditto	324	3·0	1·4	7	0
W 5, moist wheat ears 5 weeks	124	5·5	1·8	10	0
W 17, ditto	183	5·3	2·3	9	0
W 5, on wheat in the field	197	8·1	1·5	12	3

It is clear from Table V and VI that the spore dimensions of the two strains W 5 and W 17, when they are grown under similar conditions, are very much alike. On the other hand, the dimensions of the one strain when growing under different conditions differ enormously, the mean length of the spores of W 5, for example, when produced on infected wheat plants in the field being two and a half times that of spores produced in agar culture.

The two strains appear to differ little in the severity of their attack. The virulence of W 5, as seen both under greenhouse conditions and in the wheat plot in the open, has already been commented on, and W 17 proved equally virulent in the greenhouse.

Mode of Infection by *Fusarium culmorum* and *Helminthosporium sativum*

A series of experiments was undertaken to trace the course of the attack of *Fusarium culmorum* on wheat seedlings. Grain which had been sterilised by immersion in an alcoholic solution of mercuric chloride and washed in sterilised water was inoculated by dipping in a concentrated spore suspension of the fungus, after which it was sown in a number of pots of soil sterilised in the autoclave. Three or four seedlings were pulled up and examined each day starting from the third day, and in this way it was possible to gain some idea of the progressive stages of the seedling blight. Under the conditions

of the experiment, which was carried out in a heated greenhouse, no visible growth of the embryo took place until after the second day, although before that time there was considerable swelling of the whole seed following absorption of water. The fungus, on the other hand, made a rapid start and frequently there was an obvious mycelial growth on the caryopsis before the embryo started to grow out. When, as sometimes occurred, this growth covered the embryo, germination was arrested and rotting promptly followed, the seed turning brown or else bright red, due to growth and spore production of the *Fusarium*. In other instances the embryo germinated satisfactorily, but with the fungus growth on the caryopsis lying in wait for it, as it were, it failed to make much headway before penetration took place, as shown by the appearance of brown areas on the radicle or plumule. An interesting specimen was seen in which attack weakened the base of the coleoptile, so that, four days after sowing, the first leaf burst through at the point of attack instead of emerging normally at the tip. Few of the seedlings survived to push above the ground and such as did remained weak and spindly, the base of the shoot being covered with brown lesions, the roots almost completely rotted away and the caryopsis covered with a pink mass due to profuse spore production.

Microscopic examination confirmed the view that both the root and the shoot of the seedling can be penetrated by the fungus. Small seedlings were carefully removed from inoculated soil and gently washed in running water, after which the coleoptile and the cortex of the root were peeled off and examined under the microscope. Staining the tissue with cotton blue in lactophenol was sometimes resorted to as it rendered observation of the fungus less difficult. The spores of the fungus could sometimes be seen on the surface with germ tubes penetrating the cuticle of the coleoptile or the surface of the root and ramifying in the tissues below. Penetration seemed frequently to occur at the junction between two epidermal cells.

Helminthosporium sativum also was found to penetrate both root and shoot under similar conditions and the mode of entry appeared to be the same as in *Fusarium culmorum*.

Discussion

From the limited extent of this investigation, which could not be carried further owing to lack of time, it is possible only to draw the most general conclusions and to mention lines of further research which suggest themselves.

In the first place, the observations which were made sufficed to convince one that not only are the foot-rotting organisms present almost universally in the cereal-growing fields of the Cambridge

district, but that they may be there in sufficient quantity materially to affect the yield of the cereal crops. The importance of members of the genus *Fusarium* as causal agents of foot-rotting, stressed by Bennett in his work in the Northern Counties (3), is borne out by the results recorded in this paper, but, while he found the disease to be due in part to attack by *F. avenaceum*, isolations made in the neighbourhood of Cambridge have failed to show that this species is at all responsible for the symptoms of whiteheads and ear-blight. The only species of *Fusarium* proved to be of pathological importance is *F. culmorum*. The remaining organism shown to be pathogenic, namely *Helminthosporium sativum*, would seem to be of little practical significance at present.

The parasitism of the foot-rotting fungi is of unspecialised type. They can live in the soil as saprophytes, surviving there in absence of a growing host plant over considerable periods of time. Thence they may spread to the cereal plant which is sown there, living at first perhaps saprophytically on the withered leaf-sheaths or other dead tissues towards the base of the plant, then spreading into the living tissue of the plant and attacking it in parasitic manner. While the cereal plant is in a vigorous state of growth these two stages of fungal life on the host, the saprophytic and the parasitic, are perfectly distinct; but as the plant grows towards maturity and the tissue forming the base of the stem becomes moribund if not dead, they cease to be differentiated. In this condition the weakest parasites and possibly even saprophytes could cause penetration of the stem and it is probably this cause, rather than faulty surface sterilisation, that produced the varied collection of fungi isolated from cereal plants. Obviously all such fungi could not be reckoned as organisms causing foot-rot, and only those which proved able to infect wheat seedlings growing in soil were accounted of pathogenic value. *Fusarium culmorum* and *Helminthosporium sativum* were the only fungi isolated which proved pathogenic under these conditions, and it was shown that germ tubes of both these organisms were able to penetrate the actively growing roots and shoots of wheat seedlings.

In view of the unspecialised nature of the attack, it is not surprising to find that a strain of *Fusarium culmorum* isolated from wheat proves equally virulent on oats and barley; but this does not appear to be equally true for *Helminthosporium sativum* which seems to attack oats with difficulty. Further, it would appear that all the varieties of wheat commonly grown are susceptible to foot-rot, no pronounced degree of resistance having been observed in any variety. Of sixteen types of wheat growing in the grounds of the National Institute of Agricultural Botany, Cambridge, which included some common varieties as Squareheads Master, Yeoman, Little Joss, Wilhelmina, and Rivet, all were found to be suffering from foot-rot. While it is

possible that field trials, conducted on a statistical basis and extending over a period of years, might bring out significant differences in susceptibility amongst the commonly grown varieties, it seems evident that no quick and easy solution to the problem is to be found in varietal selection.

A more likely means of control is probably to be sought in the improvement of cultural practices, and in particular in the adoption of a balanced scheme of manuring, though here admittedly one is passing into the realms of conjecture since no scientific experiments with the view of attempting to correlate amount of disease with the manurial treatment applied seem to have been made. The results of a pot experiment in which differential manuring was tried, although inconclusive, suggest that wheat seedlings growing in soil with a high ratio of nitrogen to potassium may be specially liable to attack; and interesting results might be obtained if this experiment were repeated on a larger scale in pots, after which, if the method still showed prospects of success, it might be extended to plots in a field trial. In general, plants suffer more from the disease when growing under unsuitable conditions than when conditions are good, and fields that are water-logged or poor in texture undoubtedly show more severe attack than do fields in good state. From the point of view of foot-rot control, therefore, as well as from more general considerations, the importance of a sound system of cultivation, with provision of proper drainage and a suitable crop rotation, is to be stressed. It seems possible that the prevalence of foot-rots in the Cambridge district is bound up with the rotations commonly in practice in which the cereal crops play a predominant part, and that, in fields where severe infestation occurs, the growing of a lower proportion of cereal crops might prove economically more sound.

Summary

An account is given of a survey of the foot-rot diseases of cereals in the Cambridge district carried out during 1929 and 1930. This group of diseases proved universal, specimens of wheat, oats and barley showing the whiteheads symptom being found in almost every field examined and sometimes attaining serious proportions. Seedling blight due to fungal attack and ear-blight were comparatively rare.

Of the fungi isolated from affected plants *Fusarium culmorum* proved by far the most common. Two strains of *Helminthosporium sativum* were isolated from wheat plants and a third from a barley seedling. Pathogenicity tests showed these two species to be highly virulent towards wheat in the seedling stage and later to produce the symptom of whiteheads. While *F. culmorum* under greenhouse conditions attacked wheat, barley and oats with equal severity, *H. sativum* was

Fig. 1

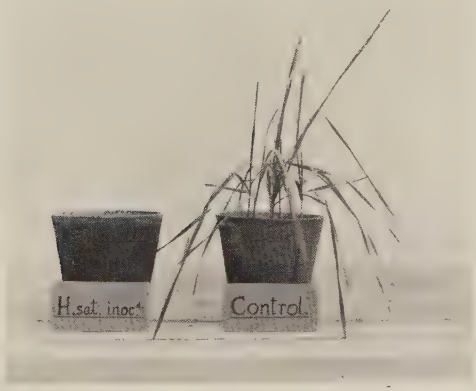

Fig. 2

Fig. 3

found to be less injurious to barley than to wheat, and hardly to affect oats at all.

Wheat seedlings growing in inoculated soil in the greenhouse were penetrated through both root and shoot tissue, the germ-tubes of both fungi making their entry by boring through the surface walls.

Observations made suggest that a sound system of farming, involving good tillage and balanced manuring, may do much to check the disease. This method appears to offer more immediate hope of success than a search for resistant varieties.

The work described here was carried out during the tenure of a Colonial Agricultural Scholarship at Cambridge. To Mr F. T. Brooks I am greatly indebted for helpful and kindly guidance during the course of the work.

EXPLANATION OF PLATE XI

Fig. 1. Pathogenicity test of *Helminthosporium sativum*. Culture W 5. Inoculated seed was sown in the two outside pots, uninoculated in the middle one. Photographed nine weeks after sowing.

Fig. 2. Pathogenicity of *H. sativum*. Culture W 5. Inoculated seed sown in the pot on the left, uninoculated in that on the right. About two weeks after sowing.

Fig. 3. The two strains of *H. sativum* isolated from wheat. On potato dextrose agar.

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SOME ORGANISMS CONCERNED IN MINE-TIMBER DECAY

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(With Plates XII–XIV and 4 Text-figures)

I. Introduction

Decay caused by fungi is now generally recognised as one of the chief agencies in the destruction of timbers in mines, and it is a commonly accepted view that the organisms responsible for the decay are Autobasidiomycetes.

An investigation of the fungi concerned in the decay of timbers of the Rand Gold Mines was started in October, 1929. A number of specimens of rotted timbers from various mines were examined and experiments started to isolate the causal organisms of the decay. The frequency with which moulds were isolated led to the suspicion that these organisms might play a more important part in the initiation of decay than has hitherto been supposed, and that they might not necessarily be the followers of the better known wood destroyers.

Acting on the suggestion of Prof. C. E. Moss, it was decided to make a study of some of these fungi and to determine, if possible, by the use of laboratory methods, their effect on wood used commonly in local mines. The results of a preliminary investigation on diseased timber from Springs Mine are here recorded.

II. Materials and methods of study

1. *Source of timber specimens*

The timber was procured in March, 1930, from 10 East No. 1 stope near the downcast No. 2 Shaft, Springs Mine, at a depth of approximately 4000 feet.

The stope is situated in the "intake" current area where the ventilation is good. The temperature in the stope averages 80° F., while the relative humidity of the air is approximately 90 per cent. Mining operations in this area had ceased some considerable time before, therefore the wood was not subjected to the usual alternation of wet and dry conditions.

2. *General condition of timber in stope*

The timber appeared sound, but when tested with the blade of a knife, a number of the props and pieces of wood lying on the ground were found to be decayed. No fructifications of Basidiomy-

cetes were found on the surface of the wood although a careful search was made for these.

Samples were chopped from one of the disused props lying on the ground and taken to the laboratory for more detailed investigation.

3. *Laboratory examination of decayed wood.*

Macroscopic observations. The surface of the wood (which has not yet been identified) was of a dusty greyish colour. Most of the inner part exhibited a white rot, but portions of the wood in the centre were still hard and appeared to be sound. Occasional black streaks could be seen in the rotted wood, running in a direction parallel to the long axis of the prop (Pl. XII, A). These black lines varied from one-eighth of an inch to half an inch in width. In a transverse section of the prop, they appeared as irregularly scattered black patches in the white rotted tissues. The outermost regions of the wood were deeply discoloured for a distance of half an inch to one inch from the surface. Narrow dense black zone lines about two millimetres wide occurred in these regions running in a longitudinal direction. Hubert (10) states that zone lines are associated with white rots only, being rare in brown rots.

Microscopic observations. Portions of the surface wood were scraped off and examined. Numerous fine hyphae and spores could be seen among the broken wood elements. The spores varied considerably in size, shape and colour, the most prominent being brown two-celled cylindrical spores about $12 \times 4\mu$.

Sections were cut through the various regions either by hand or by sliding microtome. Longitudinal sections, both radial and tangential, were on the whole more satisfactory for study than transverse sections. Delafield's haematoxylin, cotton blue with lactophenol or double staining with safranin and aniline blue as recommended by Cartwright (4), were all found to be satisfactory for showing up the hyphae in the wood.

Sections through the outer discoloured regions showed the presence of innumerable hyphae ramifying in all directions through the cells. The hyphae varied in colour and diameter, and spores like those seen in mounts from the surface material were present in the tissues.

The wood elements appeared to have suffered considerable damage. Portions of the walls and fibres had disappeared, and in the medullary rays a number of the cells were separated owing to the destruction of the middle lamellae.

In the black regions, the dominating hyphae were more uniform in size and colour being very dark and averaging 4μ in diameter. However, numerous finer hyaline threads were also observed, particularly in the vessels. The dark hyphae, which branched profusely, were present in all the elements and could be seen growing in and

between the cells of the medullary rays (Pl. XII, B). According to Thaysen and Bunker (16), the intercellular development of a mycelium necessitates the resolution of the middle lamellae of the wood tissues through the secretion of a pectosinase. Occasionally, one of the dark hyphae could be seen boring through a wall, becoming constricted as it passed through a hole smaller in diameter than itself.

A brown gum-like substance was present in the cells of the medullary rays of the black region, the amount of the substance in a cell varying from small globular drops to a mass which occluded the lumen of the cell.

In the white decayed tissues, the presence of a few extremely fine hyphae in the fibres could be determined with great difficulty. Fragments of the thick dark hyphae still persisted in a few of the elements. The general appearance of a section through the white decayed wood is clearly shown in tangential view in Pl. XII, C. The most characteristic features of the rotted wood appear to be the disintegration of the medullary ray cells and the disappearance of the middle lamellae in the cells that still persist. The walls of the fibres and their elements are extremely thin and in parts have dissolved away entirely, leaving jagged gaps.

Sections of the rotted areas treated with chlor-zinc-iodide were stained a dark blue. When treated with phloroglucin and hydrochloric acid, some of the cells of the more persistent medullary rays gave a slight lignin reaction.

Hyphae were found in the tissues of the hard, apparently sound, wood. Dark hyphae similar to those observed in the black zones were present in some of the medullary rays.

4. *Culture methods*

(i) *Isolation.*

Cultures were made by using portions of wood from the decayed white and discoloured regions and from the hard wood in the centre. Blocks roughly one inch by half an inch were cut out from the diseased wood and then sterilised.

Two methods of sterilisation were tried:

(1) by flaming the outside of the blocks;

(2) by soaking the blocks in a 1 per cent. solution of mercuric chloride and then washing in several changes of sterile distilled water.

The blocks were treated singly: and in the second method, the mercuric chloride solution was changed each time a fresh block was used. The material was handled with sterile forceps. The methods of sterilisation were found to be equally satisfactory. The outer portions of the blocks were removed with a scalpel and sections were cut from the inner parts. These sections were placed on agar plates by means

of a needle. The Petri dishes used were sterilised in a dry oven for two hours. The needles, razors and other instruments used were sterilised by passing them through a flame before each fresh section was handled.

In addition to the small sections, larger pieces of wood were gouged out from the various regions of the diseased wood and used for inoculating some of the plates. Long and Harsch (12) have pointed out that the mycelium is more likely to be present in a large piece of inoculum that in a small one. This method, however, was found to be unsatisfactory, as three or four fungi would grow from a single large piece of wood, particularly from the outer decayed portions and from the black zones, thus augmenting the difficulty of separating the organisms. As a rule only one fungus grew from the smaller sections, but in one sample two fungi were always found associated. The methods of separating these will be discussed later.

In the course of three or four days, hyphae appeared on the agar, and portions of mycelium which appeared to be pure were transferred to fresh plates.

At first, several media such as Dox's, oatmeal, potato dextrose, malt extract, and stout-wort agars were used for the cultures. Later, malt extract and stout-wort agar were employed exclusively. Both favoured a good growth of all the fungi and could be prepared readily.

The following formula for stout-wort agar was found to be satisfactory: 500 c.c. stout-wort; 500 c.c. distilled water; 2 gm. maltose; 17 gm. agar agar.

To ensure the growth of mycelium from wood which appeared macroscopically sound, the inoculum was first transferred to sterile distilled water before being planted on to the agar. Baxter (1) advocates this method particularly in wood where few hyphae are present, and where the addition of water is essential for their continued growth.

(*a*) *Establishment of pure cultures.* As has been mentioned, two of the fungi were found to grow together from one piece of inoculum. The others, however, were in the main fairly pure in that each grew from a different inoculum, but invariably they were found to be associated with bacteria which also had grown from the wood.

Schmitz (14) found that the presence of saprophytic bacteria in rotting wood very materially increased the rate of decay by fungi, the degree of this influence being found to vary with the type of bacterium present, with the species of fungus responsible for the decay and with the kind of wood attacked. However, as the present investigation was primarily concerned with the fungi, no attempt was made to establish cultures of the bacteria in order to determine what part they played in the decay.

To inhibit the growth of the bacteria and to be quite sure of

dealing with pure cultures, acidified media were tried as suggested by Brown (2) and by Hopkins (9). It was found that all the fungi grew well on agar to which two drops of lactic acid were added per 5 c.c. of the medium, before it was poured into a Petri dish.

As the fungi grew more rapidly than the bacteria, it was possible to separate the mycelial tips and transfer them to fresh acidified media. Mycelium developed normally, forming spores in the course of a few days.

Single spore cultures were established by the method of fractional plating.

(*b*) *Separation of the two associated fungi.* The mycelium grew from some of the inocula as dark grey masses of hyphae, which at a later stage appeared almost black with a faint tinge of olive. Macroscopically the cultures seemed pure, but mounts revealed two distinct types of hyphae and conidia.

Some of the hyphae were very dark and averaged 4 μ in width, others were lighter in colour and more slender, being about 1·5–2 μ wide. Both types were septate. Of the spores present, some were dark brown, two-celled, cylindrical and about 12 × 4 μ, while others were hyaline, elliptical and very small, averaging 5–6 × 2–3 μ. Both sets of conidia sometimes occurred in chains.

Preparations made from the surface growth on the agar indicated a predominance of the larger hyphae, but in mounts of the mycelium taken from the substratum, the delicate hyphae were more numerous.

Portions of the surface and sub-surface mycelium were transferred to nutrient agar plates. The method of taking the mycelium from below the surface suggested by Brown (2) for freeing fungal cultures from bacteria was employed. After a week's growth, slight differences could be noted in the cultures, but microscopical examination revealed the presence of both types of spores and hyphae.

Repeated transfers were made in the manner described above, until cultures were obtained in which one or other of the fungi predominated. Single-spore cultures of each fungus were then established by the usual method of fractional plating. As the larger spores would not germinate in a plain gelatine agar, it was found necessary to use a nutrient medium, such as malt agar, which had been carefully filtered. The separation of the spores, when both were present, was comparatively simple owing to their differences in size, shape and colour.

The fungus with the large spores was afterwards identified as *Bispora effusa* Peck*.

Bacterial colonies were present in the cultures as in those of the other fungi isolated from the wood, but were eliminated by the process already described.

* I am indebted to Dr Johanna Westerdijk for this identification.

(*c*) *Isolated organisms.* The fungi and the regions from which they were isolated are as follows:

Bispora effusa Peck (Moss 18,292)*	Black zone and inner white rotted wood.
A† (found associated with *Bispora effusa*) (Moss 18,447)	Same regions as above.
Aspergillus sp. (Moss 18,290)	Outer parts of decayed wood.
Fusarium sp. (Moss 18,289)	All regions including hard wood in centre.
B (Moss 18,291)	Outer discoloured decayed wood.
C (Moss 18,446)	All regions except hard wood in centre.

All the fungal organisms isolated were Fungi Imperfecti. No mycelium characteristic of Basidiomycetes grew from the infected wood, although frequent isolation tests were made.

Inoculation experiments were carried out on sterilised blocks of wood with the above fungi.

(ii) *Inoculation.*

Inoculation experiments were carried out primarily to determine the following issues:

(1) What part could the isolated fungi play in initiating decay in wood?

(2) Was any one of these fungi responsible for the decay phenomena exhibited in the diseased prop?

(3) Did the action of these fungi vary on different species of wood?

(*a*) *Species of wood used.* Three species of wood were selected for this study. Wattle (*Acacia decurrens*) and blue gum (*Eucalyptus globulus*) were chosen as examples of exotic timber grown in the Transvaal for mining purposes, being used extensively in local mines as props. Both seasoned and unseasoned wattle were used.

The third species, pitch pine (*Pinus palustris*), was selected as an example of imported timber. It is used chiefly for timbering shafts.

Before the experiments were started, sections of the wood were examined microscopically and found to be free from fungal hyphae. Test blocks were obtained from both the sap-wood and heart-wood of the local timbers, but sap-wood only of pitch pine was available. The blocks were cut and planed to three different sizes, the first roughly $\frac{3}{8}$ inch square, the second $\frac{3}{8} \times \frac{3}{8} \times 1\frac{1}{4}$ inches, and the third $\frac{3}{4} \times \frac{3}{4} \times 2$ inches. The smaller blocks which were more convenient

* Herbarium number in Dept. of Botany, University of the Witwatersrand, Johannesburg.

† Alphabetical letters are used to denote fungi which have not yet been identified.

for handling, were used mainly for germination tests and for studying the rate of penetration of the hyphae in the first few months. The large blocks were intended for study after a year.

Since the seasoned wattle and pitch pine material was very dry, the blocks were placed in boiling distilled water for about two minutes and then in cold distilled water to increase their moisture content. This procedure was based on a method suggested by Gerry (7).

(*b*) *Preparation of cultures and their inoculation.* Both Petri dish and tube cultures were used in the experiments.

The bottom plates of the dishes were lined with filter paper; a thin layer of cotton-wool was placed over the paper. Four of the small square blocks were put in each dish. Sufficient distilled water was added to moisten the contents of each dish. For the other cultures, cotton-wool was placed at the bottom of a tube and saturated with distilled water. A test block was inserted in each tube, and the mouth of the tube plugged with cotton-wool. The dishes and tubes were then sterilised.

The smaller blocks were inoculated by the spore suspension method. A sterilised platinum wire loop was dipped into a culture of the fungus to be used, the adhering spores being transferred to sterilised, distilled water in a small tube. This procedure was repeated until the water became cloudy with spores. The upper surface of each block was then inoculated by means of a sterilised brush which had been dipped into the spore suspension. The big blocks were inoculated either with spores or mycelium. A number of non-inoculated blocks served as controls.

The tubes were afterwards sealed with waxed paper (Burnside (3)) and placed in a culture room at a temperature of 79° F.

5. *Methods of examination*

Each day after inoculation one block from each series in the Petri dish cultures was removed and examined for germinating spores. Hand sections of the blocks were made in order to see if any of the spores had been sucked into the tissues.

For the examination of tube cultures, an individual test block was removed and examined, at intervals of approximately one, one and a half, and two months.

To be sure that the mycelium within the blocks was pure, the external hyphae were first brushed off and the surface of the block then washed in a solution of mercuric chloride. Slivers of wood from the interior were then planted on plates of malt agar. In a few days the mycelium grew out pure from the inocula.

Microtome sections of the test blocks were cut and stained.

6. *Results of inoculation of wood*

(i) *Germination studies.*

The germination tests presented some interesting features. The spores of most of the fungi germinated within twenty-four hours after inoculation on the sap-wood blocks of the various species, both seasoned and unseasoned, and a day or two later on the heart-wood blocks. The most striking results, however, were obtained with the *Aspergillus* sp. on pitch pine, for only on the tenth day after inoculation did germination begin, and then only a few spores out of many hundreds developed germ tubes.

Unfortunately, it was impossible to estimate the percentage of germination, for a number of the spores had always been sucked into the host tissues and were germinating there. Most of the spores of all the fungi, with the exception of the *Aspergillus* sp. on pitch pine. appeared to have germinated on the various species of wood.

(ii) *General growth studies.*

Surface growth. The mycelia of the various fungi grew rapidly on all the blocks except those of pitch pine whereon the growth appeared to be considerably retarded. The mycelium spread from the inoculated surface down the side of a block, and within a week the sap-wood blocks of the unseasoned wattle and blue gum were completely hidden by a dense growth of hyphae and spores. The growth on the side of heart-wood blocks and sap-wood blocks of the seasoned wattle was not so vigorous, the mycelium forming a film over the surface but not completely hiding the wood. On pitch pine the growth was confined chiefly to the inoculated surface. No growth of *Aspergillus* sp. could be observed macroscopically on this wood, even after three weeks, the appearance of the wood being as clean as before inoculation.

(iii) *Effects on wood.*

(*a*) *General account.* The results presented here were obtained from a study of the small tube cultures up to two months after inoculation.

It was evident within a week after the experiment was started that *Bispora effusa* was the outstanding fungus of the series, for it was not only growing down into the woody tissues more rapidly than the other fungi, but was beginning to traverse the elements in a horizontal direction, boring through the walls as it passed from cell to cell.

The growth of the other fungi at this stage was chiefly longitudinal in the open cavities of the vessels (or tracheids of the pine), and the hyphae had penetrated to a depth of 2–3 mm. After two months, mycelium could be located in the centre of the blocks, particularly

in the sap-wood of the unseasoned wattle and pitch pine. In the other wood, the development was not so vigorous, the growth tending to be localised near the surface. The vessels (or tracheids in the pine) contained the most mycelium. Hyphae were present to some extent in the medullary rays. Hyphae had also penetrated the blocks to from five to eight elements deep from the tangential face, the passage from cell to cell being effected through the pits.

The development of *Aspergillus* sp. in the tissues of the pine was exceedingly poor, for only a few straggling hyphae could be detected in the tracheids near the inoculated surface.

B exhibited a curious phenomenon in that it stained the pitch pine and sap-wood blocks of the unseasoned wattle a pink colour. It had secreted a red pigment in the agar cultures, but no pink coloration had been apparent in the diseased wood from which it had been isolated.

(*b*) Bispora effusa *as a wood destroyer.* The effects of *Bispora effusa* on the wood were very striking and, on the whole, appeared comparable to the decay phenomena noted in the diseased prop.

On unseasoned wattle. Sap-wood. The fluffy black mycelium grew rapidly over the inoculated surface and sides of a block and spread on to the cotton-wool, which soon turned greyish black with the numerous hyphae.

On removing the external mycelium, the whole surface of a block was found to be coloured black. The blackening extended into the interior of the block, the extent of the discoloration varying with the age of a culture. At the end of fourteen days the blackening was visible for 2 mm. from the surfaces. As invasion proceeded, more wood became discoloured, until in cultures two months old, practically the whole interior of the block was blackened, a thin strip of wood in the centre still remaining apparently unaffected. The outer parts of the blocks at this stage were extremely soft, having a cheese-like consistency.

The microscopical examination and determination of the extent and character of the penetration was considerably facilitated by the general dark colour and thickness of most of the hyphae, which could be clearly differentiated from the host tissue. As a number of younger and more delicate hyaline hyphae were also present, the staining of sections was found advisable, particularly for photographic purposes. The darker hyphae did not take up any stain, but retained their normal colour. If heat were used in the process of staining, the larger hyphae presented a collapsed appearance.

Microscopical examination showed clearly that the discoloration of the wood was mainly due to the abundant development of the dark brown, almost black hyphae, and, to the presence of a brown gum-like substance in the cells of the medullary rays. This substance

is wholly absent from the normal uninvaded wood and is probably a decomposition product of the ray cells, its formation, apparently, being associated with the invasion of the fungus.

The hyphae were present in all the elements but were particularly profuse in the medullary rays (Pl. XIII, B), sending out branches which passed into other cells through pits or bore-holes in the walls. In radial sections some of the hyphae could be seen growing between the cells of the medullary rays, following the course of the middle lamellae and ultimately causing the separation of the individual cells. A number of the hyphae within the cells grew closely appressed to the walls which had a corroded appearance.

In the outer soft areas, some of the medullary rays had disintegrated entirely, and in tangential sections the positions of these elements were indicated merely by irregular elliptical empty spaces, occasionally with a few cells still adhering to the adjacent tissues (Pl. XIII, A). Mycelium was still abundant in the remaining rays and also in the vessels, fibres, and wood parenchyma of the decayed regions, the branching hyphae growing in a longitudinal as well as in a transverse direction. Often, the hyphae could be seen traversing a number of fibres in a direct line, becoming constricted as they passed through bore-holes which were smaller in diameter than themselves. The bore-holes made by the hyphae were small, cylindrical and perpendicular to the surface of the walls.

Hiley (11), in discussing the hyphae in larch-wood infected with *Fomes annosus*, states that "in order to make the bore-holes the hyphal tips must secrete enzymes (presumably a lignase and a cytase)," and, that "when once a hypha has gained passage from one tracheid to the next, the hole which it occupies ceases to enlarge, and it may be reasonably deduced from this that the enzymes are only secreted by the apices of the hyphae."

The diseased wood was incompletely delignified. In sections from the soft region, treated with chlor-zinc-iodide, the outer layers of the walls of the fibres and vessels turned blue. On the application of phloroglucin and hydrochloric acid, the middle lamellae, and the walls of the remaining medullary rays assumed a reddish colour. It was evident that the action of the fungus was to delignify the walls from the lumen outwards.

Heart-wood. The surface development of the mycelium was not as rapid or as luxuriant as in sap-wood blocks, the growth on the sides being comparatively scanty (Pl. XIII, B). On removal from the culture tubes, however, the blocks showed the usual black discoloration.

After two months, no appreciable softening was apparent in the outer parts of the blocks.

Penetration of the hyphae was not as deep as in the sap-wood blocks nor were the medullary ray cells as yet isolated, but in every

other respect the character of the attack was the same as in an earlier stage in the sap-wood. It was apparent that the wood would ultimately decay, although the growth of the fungus was considerably slower.

On seasoned wattle. Sap-wood. The surface growth on this wood was apparently no criterion of the extent of the decay within the wood, for the development of the external mycelium was poor compared with the growth on the sap-wood of the unseasoned wattle, and yet complete delignification of the walls of the fibres and other elements had taken place for a considerable distance in the wood. A strip of wood in the centre of the block appeared to be unaffected, although hyphae were afterwards found in medullary rays and in some of the vessels. The outer parts of the blocks were characterised by the usual discoloration which, however, had a streaky appearance owing to the black colour being deeper in some parts than in others.

The outer wood was so soft that it could be easily crumbled between the fingers and it was difficult to section. Black streaks were visible macroscopically in thin sections. When these were examined microscopically, it was found that all the darker regions were heavily infected with dark brown hyphae which branched profusely in the vessels, the branches ramifying in all directions (Pl. XIII, C). Some of the parent hyphae had attained a width of 8 μ, the average width of the rest of the darker hyphae being from 4–5 μ.

A number of the hyphae in some of the vessels and fibres had become septate at short intervals and appeared to be about to form spores: others had actually formed chains of one-septate conidia in the tissues, but this was apparent only in sections near the surface.

The mycelium was characterised by the production of numerous hyaline threads which could often be seen branching from the thick dark hyphae. These threads were very fine, the broadest measuring about 1 μ in width, the others being considerably less. Numbers of these fine hyphae, which could be distinguished only with difficulty in unstained sections, ramified through the tissues in the lighter coloured patches. These areas were remarkable for the thinning of the fibre walls, some of which had been reduced from about 5·5 μ to about 1·5 μ, and also for the limited number of broad dark hyphae, which, moreover, appeared to be somewhat lighter in colour than the normal hyphae. It was obvious, however, that they had been more abundant originally, for a number of small empty bore-holes could be seen in the walls.

The limited number of the broader hyphae was probably due to the poor supply of food material in the lighter coloured areas. Hartig(8), in explaining the absence from, or the limited occurrence of, mycelium in wood in an advanced stage of decay, states that when hyphae have to traverse regions of tissue containing no

proteids their apices are supplied with protoplasm sent forward at the expense of the older parts of the hyphae. The latter are therefore soon emptied, and fill with air. Although the empty hyphae persist for some time, they finally disappear under the decomposing influence of the fungus. The consequence is that one may frequently fail to find anything of the fungus itself.

The passage of the hyphae from cell to cell, as in the seasoned wood, was in no way contingent on connecting pits, for the end of a hypha appeared to penetrate the cell wall wherever it happened to reach it. The bore-holes were extremely small and appeared to be from 1–1·5 μ in diameter. A hypha became considerably constricted, as it passed through a hole.

Attack in the middle lamellae of the medullary ray cell walls was noted in sections through the outer decayed portions of the wood. The isolation of the cells was not as complete as in the sap-wood of the unseasoned wattle.

Microchemical tests with the usual reagents indicated that the chemical changes in the decayed region were more profound than in the softened wood of the unseasoned wattle. The walls of all the elements except the medullary rays, turned dark blue with chlorzinc-iodide. Tests with phloroglucin and hydrochloric acid showed a reaction for lignin in the medullary rays and the middle lamellae of some of the walls of the fibres. Dissolution of the outer parts of the fibre walls had taken place in the light-coloured patches, for as already mentioned, a number of the walls showed an appreciable thinning, and in parts had disappeared entirely, leaving little gaps.

Heart-wood. The results obtained from studies of the growth of *Bispora effusa* on this wood were practically the same as in the heart-wood of the unseasoned wattle for the same period (Pl. XIV, A).

A number of the blocks had been cut through the centre of the timber and contained some pith tissue. As in the medullary rays (Pl. XIV, B) the pith cells were heavily invaded by hyphae which could be seen growing in and between the cells. The gum-like substance, which was present in the medullary rays, also occurred in the pith cells.

On pitch pine. After six weeks, the blocks were penetrated for 1·2 cm. from the inoculated surfaces and about 1–2 mm. from the side surfaces. The surface growth was comparatively poor, as can be seen from the photograph, the most abundant mycelium occurring on the inoculated surface.

At this stage the texture of the wood was unaltered.

The course of the hyphae within the tissues was chiefly longitudinal in the tracheids and horizontal in the medullary rays. The hyphae in the tracheids branched freely, the branches travelling in a transverse direction from cell to cell and showing the charac-

teristic constriction of the hyphae as they passed through the small bore-holes (Pl. XIV, C).

The amount of mycelium was distinctly greater in the spring than in the autumn wood. In the latter, the hyphae were comparatively few and did not branch as profusely as in the spring wood. A number of hyphae could be seen passing through the thick walls of the autumn elements (Pl. XIV, D).

No attack was observed on the middle lamellae of the cells of the medullary rays and no significant results were obtained with microchemical tests.

On blue gum. Marked differences were apparent in the surface growth on the sap-wood and heart-wood blocks, the growth of the former being as luxuriant as on the sap-wood blocks of the unseasoned wattle.

Although the whole surface of a sap-wood block was deeply discoloured, no blackening was apparent in the interior and very little penetration of the hyphae had occurred, even after two months.

Sectioning of the material was extremely difficult, owing to the hardness of the wood and to the "torse" grain. Hyphae were found at a distance of 1–2 mm. from the discoloured surface, but these were very few and were not as dark as the hyphae on the surface of the wood. Most hyphae occurred in the layers of elements adjacent to the discoloured surface and were present in most of the cells. The lumina of the medullary ray cells in this region were generally filled with a brown gum-like substance in which an occasional hypha could be seen.

The tendency of the hyphae to bore through thick cell walls was also apparent in this wood, and, again, the characteristic constriction and small bore-holes were noted.

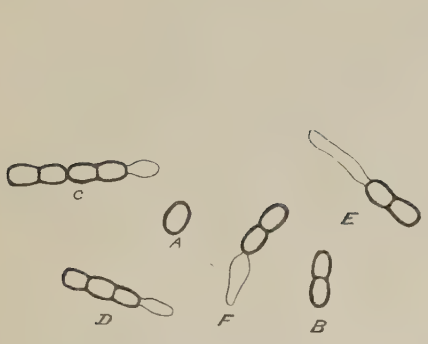
In the heart-wood blocks, internal mycelium was scantier than in the sap-wood blocks and only a few hyphae were observed in vessels and other elements near the surface.

7. *Notes on morphological and physiological characters of* Bispora effusa *Peck*

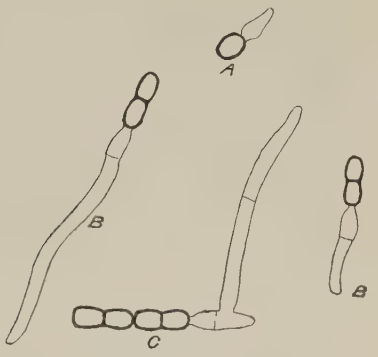
(i) *Spores and their germination in drop cultures.*

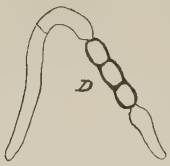
The spores vary greatly in size and shape from almost spherical about 6–7 × 4–5 μ to large cylindrical 18 × 4–5 μ (Text-fig. 1). The large conidia, which are rare, are usually three-celled. The spores most often found in cultures are uniseptate and measure 12–14 × 4–5 μ. The brownish black spores are constricted at the septa: each cell shows an oil globule.

The spores germinated within fifteen hours at a temperature of 80° F., in a nutrient agar medium, in 2 per cent. cane sugar solution

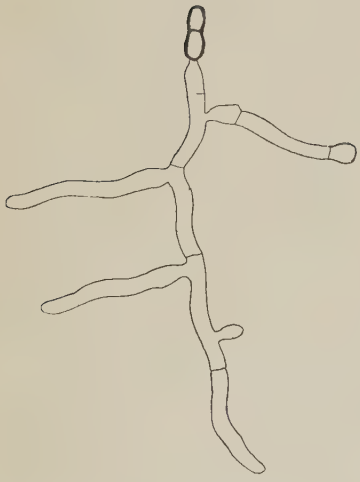


Text-fig. 1. Spores germinating 15 hours after inoculation. ×625.

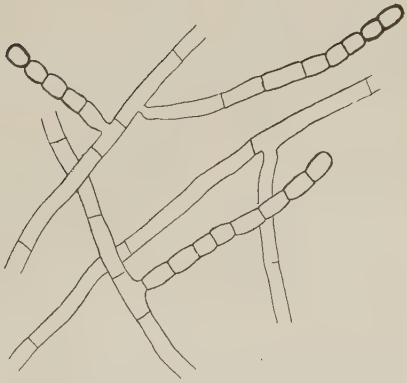




Text-fig. 2. Spores 24 hours later. ×625.



Text-fig. 3. Young mycelium, third day after inoculation. ×625.



Text-fig. 4. Portion of mycelium on sixth day showing formation of conidia. ×625.

Stages of germination of spores of *Bispora effusa*.

and in wood extracts. At room temperature, averaging 65° F., spores were found to germinate twenty-four hours later. No germination took place in distilled or tap water.

A uniseptate spore usually puts out a germ tube which is produced at the end of a cell. In the three-celled spores two germ tubes are produced one at each end of the spore. The germ tubes give rise to a branched mycelium (Text-figs. 1, 2 and 3).

(ii) *Hyphae.*

The hyphae average 4 μ in diameter and are septate at varying intervals. In colour they vary from hyaline to almost black.

(iii) *Formation of spores.*

The spores are produced in chains, of varying lengths.

Distinct conidiophores are not formed. A hypha gives rise to a branch which becomes septate at short intervals. The first spore, which may be single or two-celled, is formed at the end of the branch, the others arising in basipetal succession. Each cell of the hypha divides into two by the formation of a transverse septum. The hypha becomes constricted at a septum and its walls begin to thicken and to darken. The size of an individual spore is soon fixed by the more pronounced constriction of the hypha at the septa which ultimately form the ends of the spore (Text-fig. 4).

8. *Relation of* Bispora effusa *to the other fungi isolated*

With the view to determining the behaviour of *B. effusa* in the presence of the other fungi, inoculation experiments were carried out on sterilised sap-wood blocks of the unseasoned wattle, which was favourable for the growth of all the fungi. Some of the blocks were inoculated with the spores of *B. effusa* and A, which had been isolated together, while others were inoculated with the spores of all six fungi.

After two months, examination of the blocks inoculated with the two fungi indicated that the presence of A had in no way inhibited the activity of the *B. effusa*, for its effects on the wood were the same as in pure cultures. The usual discoloration was apparent in the interior of the wood and the characteristic mycelium of *B. effusa* could be seen in the medullary rays and other elements. The hyphae of A were chiefly confined to the vessels near the inoculated surface and could be clearly distinguished from those of the other fungus by the colour and size.

In the other experiment, no surface development of *B. effusa* could be seen, although the other fungi appeared to be normally active, B almost covering the surface of the blocks, and the other fungal growths, with its white mycelium, and yellowish white spores. On

microscopical observation, hyphae could be found in the vessels, but none were of the *Bispora* type.

It seems that certain of these fungi exert a toxic influence on the spores or young germ tubes of *B. effusa*. No definite evidence of this can be given until further experiments are carried out. However, as *B. effusa* was alive in the diseased prop and was isolated from regions in which the other fungi were also present, it would seem that it was the primary fungus in the wood, and once it had established itself, could not be affected by the other fungi which apparently followed later.

III. Discussion

In surveying the literature on wood-destroying fungi, very few references could be found to Fungi Imperfecti as the causal organisms of decay.

Marshall Ward (18) was the first to point out the possibility of *Penicillium* initiating and carrying on the destruction of wood. Gerry (7), working on certain moulds and their penetration into wood, found, by artificial inoculation experiments, that *Penicillium divaricatum* Thom and *Monilia sitophila* (Mont.) Sacc. were capable of penetrating the cell walls of spruce, aspen, and red oak, and that the former caused an appreciable softening of the wood after two years' culture.

Siggers (15) noted that logs of white ash (*Fraxinus americana*) and yellow poplar (*Liriodendron tulipifera*), heavily infected with *Torula ligniperda* Willkomm, had decreased in strength although the cell walls of the attacked wood were seldom perforated by the mycelium of the species.

Tuzson (17) states that *Bispora monilioides* Corda attacks freshly felled logs of beech, causing a white rot.

Corda (5) described several species of *Bispora*. He pointed out that they occur on rotted logs, but did not state whether they are responsible for the decay of the wood.

It is evident from the results here recorded that Fungi Imperfecti are capable of infecting healthy wood and that a fungus such as *B. effusa* can cause serious damage to certain timbers within a short period. Whether the other fungi affect the strength of the wood cannot yet be determined, and cultures are being kept for further observation.

The comparative study of *B. effusa* on the different species and parts of woods indicates that there is considerable variation in the rates of growth and penetration into the tissues. The seasoned wattle appears to be most susceptible and the blue gum the most resistant to attack, although invasion of the walls of the cells is also apparent in the latter.

From observations on the behaviour of *B. effusa* in pure culture,

it seems reasonable to assume that it is the fungus which is responsible for the decay in the diseased mine prop.

The black discoloured streaks in the diseased wood, the size and colour of the hyphae, and their constriction in passing through bore-holes, the isolation of the medullary ray cells through the attack on the middle lamellae, and the delignification of the walls of the other elements all indicate that *B. effusa* is the causal organism of the decay.

According to Mitchell (13), Basidiomycetes are responsible for all fungal decays of wood in mines. No experimental evidence, however, is given in support of this statement.

Fritz (6) has pointed out that the presence of a sporophore on the exterior of decayed wood does not necessarily mean that this fruiting body has been produced by the mycelium causing the decay; also that identification on the basis of the characteristics of the attacked wood is not a guarantee of the identity of the causal organism, for it is conceivable that different types of fungi may cause similar changes in an attacked wood. How far apart these types may be is seen from the fact that in a superficial or even detailed microscopic examination of wood attacked by *B. effusa*, all the characteristics previously thought to be typical of wood-destroying Basidiomycetes are displayed. Thus, Hubert (10) and Thaysen and Bunker (16), point out that an indisputable evidence of decay is revealed in microscopic examination of wood by the presence of bore-holes or perforations of the cell walls by the hyphae of wood-destroying fungi. The structure of the bore-holes, according to Hubert, is frequently typical for the particular Basidiomycete producing them. He illustrates, among others, the nature of the perforations produced by *Trametes Pini*. These, however, are identical with the bore-holes caused by the hyphae of *B. effusa* (Pl. XIV, D).

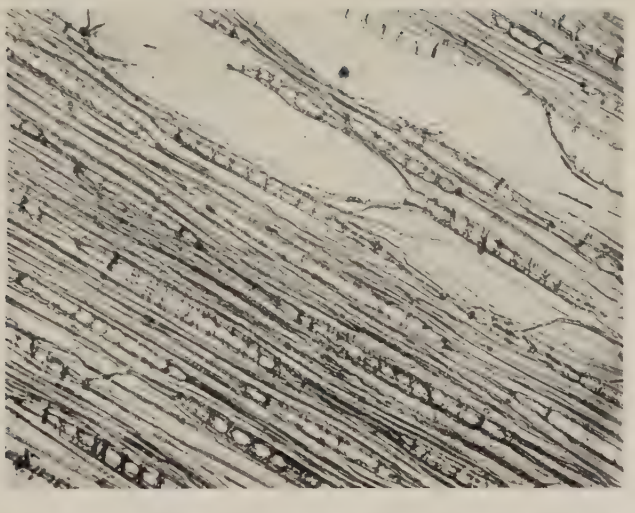
It is only by carrying out Koch's postulates in connection with these fungi that definite and reliable criteria are available for the establishment of the identity of causal organisms of decay.

In mines recognition of decay of the timbers is generally afforded by the presence of fruiting bodies of Basidiomycetes. The presence of such a fungus as *B. effusa* in mine timbers must be regarded as extremely important, since the gross appearance of the wood is unchanged. Such a case, naturally, would be particularly dangerous, since the wood, if used for strengthening purposes, as in mine-roof construction, may suddenly and unexpectedly collapse.

IV. Summary

1. Diseased timber was procured from 11 East No. 1 stope near No. 2 Shaft, Springs Mine.

2. Six species of Fungi Imperfecti, including *Aspergillus*, were isolated and grown on various media.

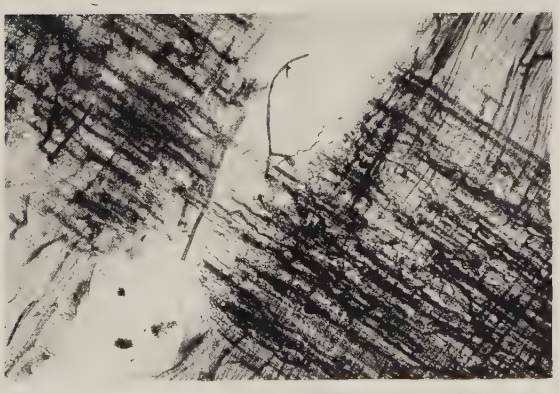


C

B

A

A



B

C

A

B

C

D

3. These were separately inoculated into wood blocks.
4. Germination and growth phenomena were carefully compared.
5. Of these fungi, *Bispora effusa* Peck was found to exhibit the characteristics of a wood-destroying organism in a comparatively short time.
6. The activities of this fungus included penetration of the walls of the wood elements within ten days. After some time the hyphae were observed to attack the middle lamellae of the medullary ray walls, causing the separation of the individual cells.
7. A complete softening of wood to a pulpy consistency occurred in sap-wood of the seasoned and unseasoned wattle in six weeks.

The work on which this paper is based, was carried out in the Department of Botany, University of the Witwatersrand, Johannesburg, under the direction of Prof. C. E. Moss. The writer wishes to record her thanks to Prof. Moss and Dr E. M. Young for kindly advice and criticism throughout the investigation.

Acknowledgments are also due to the Co-operative Exchange Yards for supplying timber, and to the Government Research Grant Board for aid in meeting expenses incurred in the study.

EXPLANATION OF PLATES XII-XIV

Photographs kindly taken by Mr W. Paff, University of Witwatersrand

Plate XII

A. Vertical section through portion of diseased mine timber, showing black streaks in white decayed wood. Natural size.
B. Radial longitudinal section of black zone showing invasion of medullary rays and other elements by dark hyphae. *a*, hyphae following course of middle lamellae; *b*, drops of gum in cells. ×220.
C. Tangential longitudinal section of white wood. Note the disappearance of the middle lamellae of the walls of the medullary ray cells and the destruction of some of the other elements. ×220.

Plate XIII

A. Tangential longitudinal section. Unseasoned wattle sap-wood infected with *Bispora effusa*. Note the heavy invasion of the medullary rays and fibres at *a*. *b*, hyphae passing through fibre walls; *c*, medullary rays destroyed by fungus. ×200.
B. Radial longitudinal section of the infected unseasoned wattle, showing a medullary ray infested with hyphae. ×200.
C. Tangential longitudinal section of seasoned wattle (sap-wood). *a*, colourless hyphae ramifying through a vessel; *b*, fibres heavily invaded by hyphae; *c*, gap left by dissolution of fibre walls; *d*, hyphae passing through walls of fibres. ×200.

Plate XIV

A. Tangential section of seasoned wattle (heart-wood) infected with *Bispora effusa*. ×190.
B. R.L.S. Same as A. Medullary ray showing hyphae following course of middle lamella (denoted by arrow). ×570.
C. R.L.S. Pitch pine, inoculated with *Bispora effusa*. ×190.
D. R.L.S. Pitch pine, showing passage of a hyphae through the walls. Note constriction of hypha and small bore-holes. ×380.

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OBSERVATIONS ON *CUCURBITARIA LABURNI* (PERS.) DE NOT.

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(With 5 Text-figures)

Introduction

Fructifications of *Cucurbitaria Laburni* de Not. are of frequent occurrence on the dead branches of laburnum trees. The mycelium penetrates all the tissues, but is especially abundant in the xylem, where it forms densely tangled masses in the lumina of the vessels. The black perithecia are borne in groups on stromata, which burst through the bark.

Branches and twigs affected by the fungus may also show various types of pycnidia, and some of these are very closely associated with the perithecia. In the past there has been much discussion as to how many of these pycnidial types really belong to the life history of *Cucurbitaria Laburni*.

The fungus is usually confined to twigs and small branches on the lower parts of the tree, and these have probably died from overshading. Only two trees have been observed in which the fungus covered large main branches. One of these trees was completely dead, but the second one, observed two years ago, bore other healthy main branches, which are still unaffected. When laburnum branches are removed from healthy trees, and are left lying in the open, they become covered with fructifications of *Cucurbitaria*. A report from Elgin states that of the various types of pea sticks used, those of laburnum invariably become covered with *Cucurbitaria*, although pea sticks of other kinds do not show this fungus. It does not occur on other trees, and has not been observed on laburnum trees in London, although it is common in Cambridge and Edinburgh. Three dying laburnums in London have been kept under observation; all are now dead, but none shows *Cucurbitaria*.

This investigation was carried out in order to determine the extent to which *Cucurbitaria Laburni* may be regarded as a parasite. Also, the various types of fructifications were studied on the twigs and in culture, and experiments were made to determine how many of the pycnidial forms represent part of the life history of *Cucurbitaria Laburni*.

Fructifications Associated with *Cucurbitaria Laburni*

(i) *Literature*

Tulasne (1863) described and figured perithecia and four types of pycnidia. The following summary, modified from de Bary's (3) account of Tulasne's work, represents their order of appearance in centrifugal succession, the earliest formed being at the centre of the stroma.

1. In the middle of the stroma one or a few relatively pale coloured pycnidia, producing colourless, thin-walled, unsegmented, ovoid, cylindrical spores (5–10 × 3·5–5 μ).

2. Numerous pycnidia with thick black walls in which are abscissed:

(*a*) Colourless spores of very unequal size.

(*b*) Spores with dark brown walls, non septate, or with one transverse wall, closely resembling the spores of No. 1 in form and size.

(*c*) Brown spores like the last, but plurilocular, compound (20–30 × 7–10 μ).

3. Perithecia whose asci contain compound ascospores, which resemble 2 (*c*).

Tulasne stated that each conidial form usually occurred in its own pycnidium, but that pycnidia containing mixtures of spores were to be found. He also reported that the pycnospores were sessile, but de Bary made mention of short sterigmata.

Saccardo (1882) included two types of pycnidia in his description of *Cucurbitaria Laburni*. One had brown, uniseptate spores and was called *Diplodia Cytisi*, and the other, referred to as *Hendersonia Laburni*, had large brown compound spores.

Tubeuf (1886) claimed that *Cucurbitaria* possessed the types of fructifications shown in the following summary:

A. Pycnidia.

1. White, unicellular conidia (2·05 × 1 μ) on long conidiophores.
   - (*a*) Free on the stroma.
   - (*b*) Enclosed in cavities in the stroma.
     - (α) In cavities not yet resembling pycnidia.
     - (β) In cavities with firm dark-coloured periphery.
   - (*c*) In dark-coloured free pycnidia with coarse pseudoparenchymatous peridium.
2. Brown, unicellular, round conidia (2 μ) in small brown pycnidia.
3. Brown, plurilocular, compound conidia (20 × 8 μ).
   - (*a*) In brown smooth very large pycnidia.
   - (*b*) In darker smaller pycnidia with beaked pores.

4. Brown, bicellular, compound conidia (20 × 8–10 μ) in small dark pycnidia (*Diplodia Cytisi*).

B. Perithecia, dark with sunken pores, whose asci contain brown pluricellular, compound ascospores (28–36 × 14 μ).

Tubeuf also obtained an *Alternaria* type of spore in his cultures of *Cucurbitaria*, and from the results of inoculation experiments he added *Fusarium* as yet another spore type. Both *Alternaria* and *Fusarium* are to be regarded as contaminations, and need not be considered in the life history of *Cucurbitaria*.

Winter (1887) in Rabenhorst's *Kryptogamen Flora* quoted Fückel's description of a spermogonium form with colourless spores and a pycnidial form with brown spores of the *Diplodia* type.

Brefeld (1891) quoted the descriptions of Tulasne and Tubeuf, but challenged the latter's statement of the extreme pleomorphy of the fungus. Brefeld himself only encountered perithecia on the twigs and small black pycnidia in cultures. The pycnidia contained small ovate, colourless spores, which he regarded as identical with the type 1 of Tulasne.

Welch (1926) described *Cucurbitaria Laburni* in his monograph on North American species of *Cucurbitaria*. He recorded only perithecia and has never encountered any pycnidial stage.

In the present investigation, perithecia and two types of pycnidia have been encountered. The latter will be referred to as pycnidia A and pycnidia B. Observations have also been made on fructifications of other fungi associated with *Cucurbitaria* on laburnum twigs.

(ii) *Observations on the fructifications of* Cucurbitaria Laburni

(*a*) *Perithecia.*

Perithecia occur in black pustules on the twigs. They were produced on one inoculated tree, but have never been obtained in cultures. Each perithecium has a depression at the apex, and contains sterile paraphyses and asci. There are four to eight large muriform, brown spores in an ascus, which are shot forth violently to a height of at least half an inch, and may be collected by supporting sterile cover glasses over perithecia kept damp in a Petri dish. If the cover glass is focused under the microscope, it is observed that the spores are shot off in groups of three to eight. These represent the spores from an individual ascus, and they are often widely separated on the cover glass, thereby indicating that the spores escape from the ascus one by one and not as one mass. The actual process of spore discharge takes place as described by Tubeuf.

The perithecia are about 0·75–1 mm. in diameter, the asci measure about 200 μ before elongation, and the ascospores are 24–34 × 12–16 μ. Tulasne and Tubeuf both gave figures of the perithecia.

On germination the ascospores swell very considerably, and each cell of the compound spore may give rise to a germ tube. These germ tubes become septate and branch profusely. Fusions between hyphae from the same spore and from different spores are common. No spores were formed by the hyphae in hanging drop cultures. The fungus was cultivated on the following media: Dox's agar, asparagin agar, potato agar, beer-wort agar, laburnum-wood-extract agar, and also on laburnum wood blocks. A whitish brown, cottony type of mycelium is produced on laburnum media, and the fungus shows characteristic types of growth on the other media. All media become discoloured, especially if the cultures are kept in direct light. Pycnidia A are produced on laburnum media, and pycnidia B are produced on all media especially when kept in the light. Attempts to obtain perithecia in cultures have been unsuccessful.

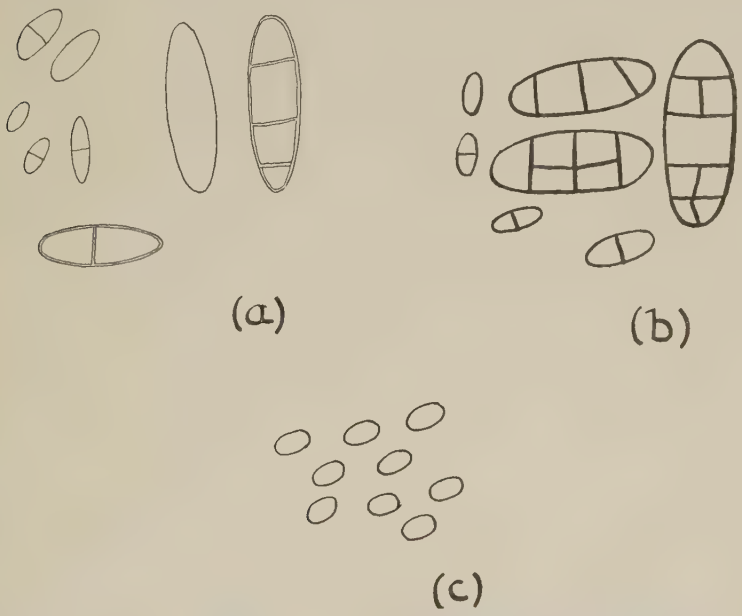
(*b*) *Pycnidia A.*

Pustules of black pycnidia containing large brown, muriform pycnospores occur on the twigs. These pycnidia have no depression at the apex, but otherwise they resemble the perithecia exactly in size and form, and very often there are black pustules containing a mixture of pycnidia and perithecia. The pycnospores are 16–24 μ long (usually 20 μ), and 8–9 μ broad, and are indistinguishable from the ascospores. They are formed directly from the innermost cells of the pycnidial wall, and there are no conidiophores (see Tulasne's figures (5)). As the spores become detached they form a dense mass in the pycnidial cavity, which gradually enlarges as more and more spores are formed from the wall.

Under wet conditions the pycnospores exude passively from the pycnidia as black shining drops. Their behaviour on germination and in culture is identical with that of the ascospores. Moreover, since this type of pycnidium is formed in cultures from ascospores on laburnum agar, there is no doubt that it belongs to *Cucurbitaria*. It is referred to as pycnidium A, and corresponds to No. 2 (*c*) of Tulasne, and No. 3 of Tubeuf. Both Tulasne and Tubeuf gave figures. When they are detached from the pycnidial wall the young spores are usually large, colourless and unicellular. However, first year pycnidia may show detached spores, which are extremely variable in size, colour and form, ranging from small ovoid unicellular spores, which are brown or colourless, through brown or colourless bicellular spores to larger brown or colourless spores with varying numbers of septa (see Text-fig. 1 *a*, *b*). Some of these detached spores have relatively thick walls and are presumably mature. It seems probable that they correspond to Tulasne's types 2 (*a*) and 2 (*b*), and that these latter really represent incompletely developed forms of his type 2 (*c*). Even comparatively old pycnidia often show a

small percentage of brown and colourless spores of the various types.

When formed in cultures the walls of pycnidia A may be very rudimentary.



Text-fig. 1. *a*. Colourless spores from a young pycnidium, type A. *b*. Brown spores from the same. *c*. Spores from a pycnidium, type B. ×1000 approx.

(*c*) *Pycnidia B.*

Small black pycnidia about $\frac{1}{4}$ mm. to $\frac{1}{2}$ mm. in diameter, containing masses of colourless, unicellular conidia with blunt ends, are extremely characteristic of the cultures (see Text-fig. 1 *c*). The spores measure 4–6 × 3 μ, and they are produced without conidiophores, as are pycnospores A. These pycnidia are referred to as pycnidia B, and they belong to *Cucurbitaria*, because their behaviour in culture is identical with that of ascospores, and because they themselves are produced in cultures from ascospores.

Pycnidia containing a mixture of pycnospores A and B are common in cultures and on the twigs. Such pycnidia are mature, and the colourless spores B are not to be confused with the young colourless stages of spores A, which are usually much larger than spores B. On twigs the first formed pycnidia at the centre of the pustule are typically paler coloured, and contain a mixture of spores A and B, whereas the later formed pycnidia contain spores A only. Occa-

sionally, a pycnidium containing spores B only has been found on a twig.

Pycnidia B correspond to Brefeld's culture pycnidia, and to Tulasne's pycnidia type 1. They were figured by Tulasne (*loc. cit.* Pl. XXVII), and according to him the pale coloured pycnidia on the twigs contain colourless spores only, whereas my pale coloured pycnidia on twigs more frequently contain a mixture of spores A and B. Pycnidia containing colourless spores only are more typical of the cultures.

Since pycnospores B often occur in one and the same pycnidium as mature pycnospores A, and since pycnidia B are particularly characteristic of the cultures, it seems probable that the spores B represent an incompletely developed form of the brown muriform pycnospores A. These incompletely developed spores are, however, capable of germination.

(iii) *Observations on other fungi which are associated with* Cucurbitaria *on laburnum twigs*

(*a*) *Phomopsis rudis.*

Laburnum twigs bearing perithecia of *Cucurbitaria Laburni* very frequently show another typical pycnidial fungus. The bark is yellowish, and bears numerous closely crowded, small black stromata, rarely more than $\frac{1}{2}$ mm. in diameter, which eventually puncture the bark. Each stroma contains one or more cavities lined with long conidiophores bearing long, colourless, unicellular conidia, which have a characteristic oil drop at each end. These conidia measure 5–10 × 2–3 μ and the conidiophores are about 20 μ long. The spores often emerge from the pycnidia in reddish, or yellowish white coiled tendrils. This type of pycnidium often appears on twigs which have been inoculated with ascospores of *Cucurbitaria Laburni*, and it is followed later by pycnidia A.

These pycnidia agree with Tubeuf's figures and descriptions of pycnidia type 1. His measurements of spores (2·5 × 1 μ) are smaller than mine, but the spores show great variation in size. Also, Tubeuf's measurements are not always reliable, because he described an ascus 121 μ in length, which was much too small to contain his 8 ascospores 28–36 μ in length. Tubeuf regarded his pycnidia 1 as a spore stage of *Cucurbitaria Laburni*, and considered that Tulasne's pycnidia with colourless spores were really of this type, in spite of the difference in shape, and in spite of the fact that Tulasne's spores were sessile. As has already been pointed out, Tulasne's pycnidia correspond to my pycnidia B.

Although Tubeuf's pycnidia seem to be closely associated with *Cucurbitaria Laburni*, they are never found in the same pustule at the

same time as perithecia. Also, cultures made from the spores do not resemble those made from ascospores of *Cucurbitaria*. Moreover, when twigs were inoculated with mycelium from these cultures *Cucurbitaria* pycnidia A and B were not produced, and the twigs merely became covered with Tubeuf's pycnidia. Hence the pycnidia in question cannot be regarded as belonging to *Cucurbitaria Laburni*. They were identified by Mr E. W. Mason as belonging to *Phomopsis rudis*, of which the perfect stage is *Diaporthe rudis*. They correspond to the description of *Phoma rudis* (*Phomopsis rudis*) in Rabenhorst's *Kryptogamen Flora* (9).

(*b*) *Fungal hyphae in lenticels and dead snags.*

One other type of pycnidium was obtained by plating out the hyphae, which occur in the lenticels of laburnum and also in the dead snags of the control twigs of field inoculations. The mycelium bears considerable resemblance to that of *Cucurbitaria Laburni*, but it develops small black pycnidia ($\frac{1}{4}$–$\frac{1}{2}$ mm.) with small ovoid brown spores (4–6 × 3–4 μ) which have no apparent conidiophores. These pycnidia probably belong to *Coniothyrium Laburni*, which is mentioned by Saccardo (4*a*) as occurring on laburnums.

(iv) *Discussion of the fructifications of* Cucurbitaria Laburni

Thus *Cucurbitaria Laburni* possesses perithecia and two types of pycnidia. The perithecia agree with those described by other authors. The pycnidia with muriform pycnospores (A) correspond with those of Tulasne and Tubeuf, and are mentioned by Saccardo. The pycnidia with colourless spores (B) are identical with those of Tulasne and Brefeld.

The other pycnospores mentioned by Tulasne, viz. 2 (*a*) and 2 (*b*) probably represent incompletely developed forms of pycnospores A. Such incompletely developed forms have been encountered in pycnidia A in this investigation. The *Diplodia Cytisi* of several authors may possibly also represent young stages of A, but it is more likely that they were dealing with a distinct fungus. Tubeuf's pycnidia with colourless spores belong to *Phomopsis rudis*, while his pycnidia of type 2 have not been encountered. The mycelium obtained in cultures from his pycnospores 2 was different from that obtained from ascospores of *Cucurbitaria*, and hence here again he was probably dealing with a distinct fungus. The "Fungus Spermogonium" mentioned in Rabenhorst's *Kryptogamen Flora* may have represented pycnidia B, or was possibly identical with *Phomopsis rudis*.

Inoculation experiments on the laburnum

(i) *Literature*

Tubeuf considered that *Cucurbitaria Laburni* was a wound parasite, and was capable of penetrating and spreading in living branches, thus killing the trees. He described a plantation of laburnums in an ornamental garden at Munich in which nearly all the trees had branches covered with *Cucurbitaria*. He considered that under natural conditions *Cucurbitaria* enters the laburnum through a wound, and the most typical wounds with which he had to deal were those made by hailstones. The wood in the neighbourhood of a wound is brown, and the fungus lives on the brown wood. Meanwhile, the browning ("Verkernung") spreads, because it is a reaction of the host to the fungus, and is occasioned by the secretion of enzymes or of some metabolic product on the part of the latter. The hyphae follow in the wake of the browning and live on the decomposition products of the brown wood.

So convinced was Tubeuf of the destructive action of *Cucurbitaria Laburni* on laburnums that he recommended control measures, which included the covering of wounds, and the removal and destruction of all diseased branches.

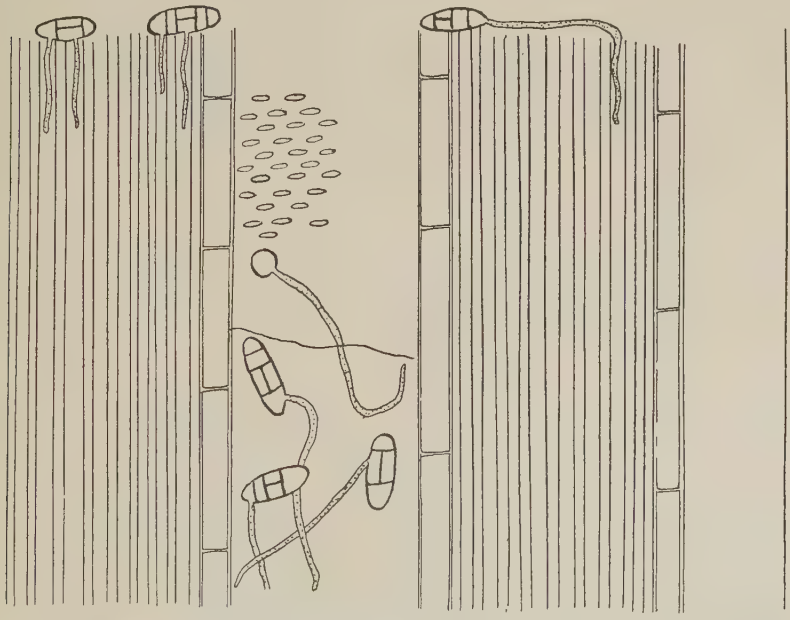
Tubeuf's descriptions of the extent to which the trees in the plantation showed *Cucurbitaria* would certainly indicate that the fungus is a wound parasite, but the evidence from his inoculations is not at all convincing. He removed from his garden a young tree, which had several dead branches covered with *Cucurbitaria*, and the trunk and branches showed several hailstone wounds associated with *Cucurbitaria* mycelium. He transferred the tree to a room, and watched the gradual spread of the fungus accompanied by the drying up and dying of all the upper parts of the tree. However, this may very well have been occasioned by transplanting, and had the tree remained in the garden it is doubtful whether *Cucurbitaria* would have caused the damage. Tubeuf also made inoculations on healthy trees by placing a spore suspension in a slit made in a branch, or by inserting a splinter of wood riddled with mycelium into a notch in a healthy branch. He reported that the autumn inoculations caused a browning of 2–6 cm. by the spring, and that the spring inoculations caused a browning of 10 cm. by the autumn. The hyphae spread for 3–4 cm. and produced fructifications. He did not mention any further progress of the fungus, nor does he report whether the disease spread into the neighbouring internodes and caused a die back.

In the present investigation inoculations were made on cut twigs in the laboratory and also on trees in the field. The laburnums used were all varieties of *Laburnum Anagyroides* Medik. (*Laburnum vulgare*

J. Presl), which corresponds to the old *Cytisus Laburnum* Linn., the name used by Tubeuf. For the spore inoculations a suspension of spores in water was used, and the spores were obtained from perithecia and pycnidia kept damp in a Petri dish. The ascospores were collected on sterilised slides supported above the fructifications, and the pycnospores were collected with a brush from the shining black drops exuding from the pycnidia. For the mycelium inoculations cultures of *Cucurbitaria* on beer wort were usually employed. Inoculations were usually made through wounds of various kinds, and controls were also made in which the twigs were wounded but not inoculated. The cut ends of twigs were either tied up with tinfoil and wool or left exposed, and cuts in intact branches were tied up with wool or covered with grafting wax.

(ii) *Inoculations of cut twigs in the laboratory*

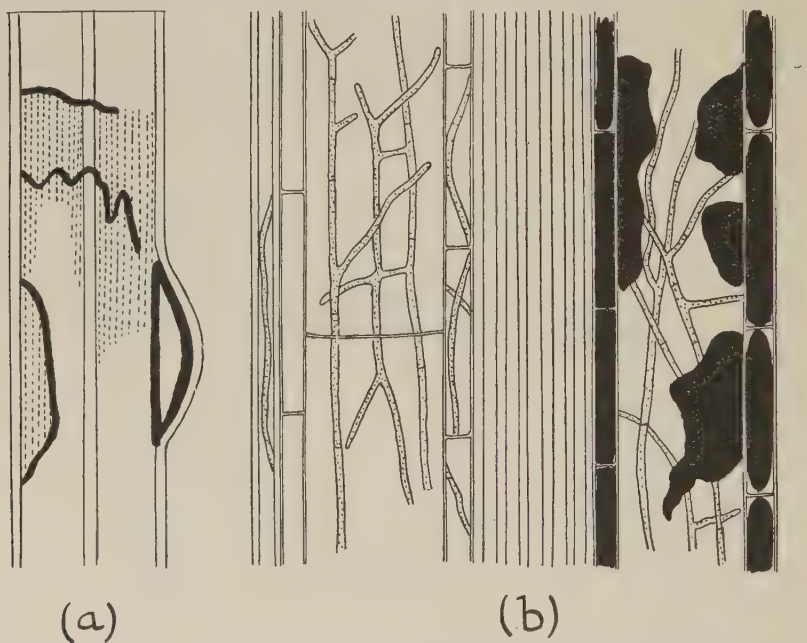
Twigs were inoculated in the laboratory by inserting mycelium or a spore suspension into cuts made in the side of a twig, or by cutting off the end of a twig and placing a spore suspension on the freshly exposed surface. The twigs were kept with their ends in water or in damp sand. In all, *Cucurbitaria* invaded the whole of the twig and pycnidia A appeared after about three to four months. It was also



Text-fig. 2. Spores germinating on the surface of an inoculated twig, and also in the vessels. × 500 approx.

possible to cause invasion of the twig from the cut stumps of short shoots bearing flowers.

Sections through inoculated surfaces were cut at various intervals after inoculation. After one or two days some of the spores had germinated on the surface, and had sent hyphae down into the elements of the xylem, while others had got sucked down into the vessels (sometimes as much as $\frac{1}{4}$ inch), and had germinated there (see Text-fig. 2). Brooks and Moore(2) found that in the invasion



Text-fig. 3. *a*. Diagram of a section of a twig inoculated in the laboratory, showing the black (black) and blackish brown (dotted) regions; the rest of the twig is pale brown. $\times \frac{1}{2}$. *b*. Diagram of a section in the blackish brown region showing hyphae in the vessels, fibres and parenchyma, and gum (black) in the vessels and parenchyma. $\times$ 500 approx.

of freshly cut plum twigs by *Stereum purpureum* the small spores were sucked down into the vessels where they germinated. They deduced that the cut end of the twig sucked in the water of the spore suspension, and that the spores were carried in quite passively. Now the spores of *Cucurbitaria* are very large compared with those of *Stereum*, and the fact that many of these large spores are carried into the vessels with the water of the suspension indicates that there must be a considerable suctional force at the freshly cut end of a twig.

The hyphae of *Cucurbitaria* penetrated to about one quarter to half an inch in two months, and to about one inch in three months

The invaded wood became blackish brown, owing partly to dense accumulations of dark coloured hyphae in the vessels, and partly to masses of brown gum, which were especially abundant in the wood parenchyma and the medullary rays (see Text-fig. 3). Hyphae extended beyond the limit of blackish browning, but stopped short of the limit of pale browning due to gum alone. In inoculations made in the side of a twig the hyphae spread more rapidly in a longitudinal direction than in a transverse direction. The twigs usually became contaminated with *Phomopsis rudis*, and pycnidia of the latter often preceded pycnidia A. However, the above appearances of infected twigs were also characteristic of twigs bearing pycnidia A, but not showing *Phomopsis* pycnidia, and hence they may be attributed to *Cucurbitaria* and not to *Phomopsis*.

None of the laboratory twigs could be kept in an actively growing condition, so that their invasion by *Cucurbitaria* is no evidence for its parasitic action on trees.

(iii) *Inoculation experiments on trees*

The majority of the trees inoculated in the field were young, and were growing in a well-lighted open situation. A few larger trees growing in the shade were used, the lower branches being inoculated. Inoculations were made at all seasons of the year for seventeen months (Nov. 1928 to March 1930).

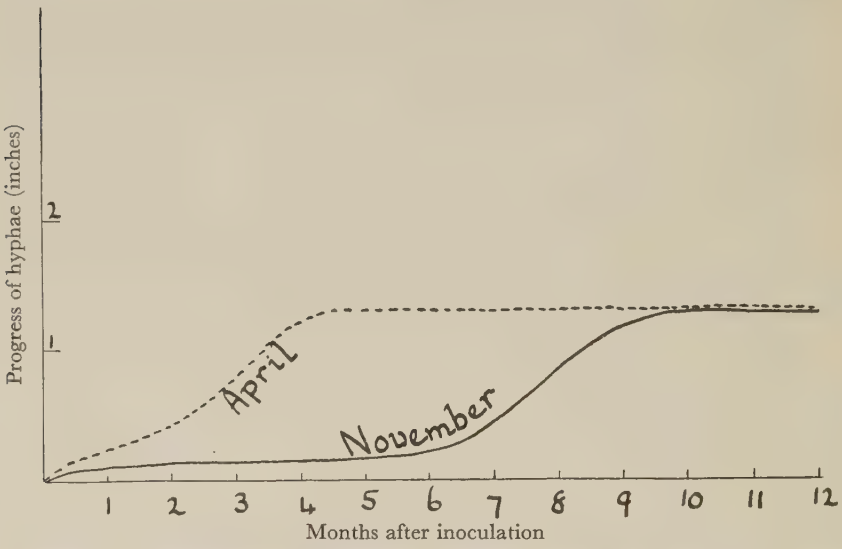
Inoculations of unwounded surfaces such as internodes and leaf scars were entirely unsuccessful. So also were inoculations through wounds made in the sides of intact branches; even if the mycelium was inserted in a cut made in the main trunk, no infection occurred. The hyphae and browning formed only a limited zone about the cut, and did not seem to spread.

Branches and twigs were cut off with pruning secateurs, and the exposed surfaces were inoculated. Some of these cuts were made at the level of a node, and in these the spores germinated, and sometimes produced pycnidia A on the exposed surface, but the hyphae and browning penetrated only a negligible distance.

Other twigs were cut so that snags were left above the node similar to those often made in pruning. During the summer months both the inoculated and control snags withered back to the next living node, and in the inoculated twig the fungus rapidly spread through the dead snag, and usually produced pycnidia A. The wood in the inoculated snags was pale brown and dry, with thin black streaks due to accumulations of dark coloured hyphae in the vessels, and gum was present, especially in the wood parenchyma and the medullary rays. The control snags became invaded by various fungi; *Phomopsis rudis* was very common, and so also was a species of *Nectria*. Sometimes the controls became spontaneously infected by *Cucurbitaria*.

Cucurbitaria never spread through the snag until after it had withered back to the node in the summer months. Hence there was a considerable delay in the spread of the fungus in inoculations made in late summer, autumn and winter, while inoculations made in spring and early summer resulted in a rapid invasion of the snag (see Text-fig. 4). *Cucurbitaria* was also capable of growing in snags which had been exposed some time before inoculation.

Both the control and inoculated snags produced cork barriers at the base in the cortex and phloem, and here there was also a considerable accumulation of gum in the xylem (see Text-fig. 5). There

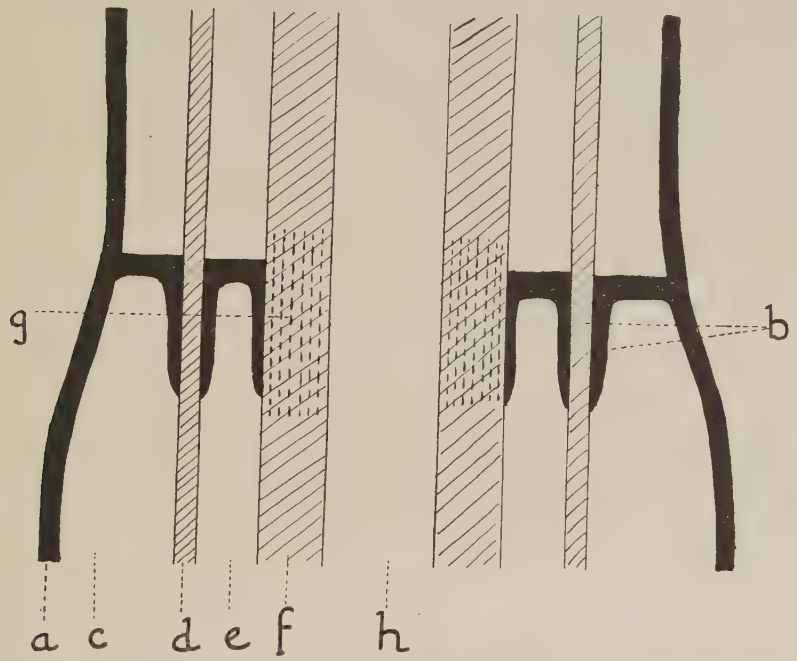


Text-fig. 4. Graphs showing the progress of hyphae in snags inoculated in the winter and spring respectively.

was no cork barrier in the pith. *Cucurbitaria* did not penetrate below the node, and out of nearly one hundred and fifty inoculations there have been only about a dozen examples of the dying back of the twig below the snag. Even these did not always show *Cucurbitaria* pycnidia, and they would probably have died in any case, since they were usually on shaded lower branches. Moreover, there have been several examples of control twigs dying on shaded lower branches. Even inoculations through large snags (about two inches in diameter) did not result in penetration of the fungus from the snag.

Hence, *Cucurbitaria* is not parasitic on trees of normal vigour, but the results from shaded trees indicated that *Cucurbitaria* would probably spread if the tree were unfavourably circumstanced. An inoculated tree was cut down in Dec. 1929, and in the course of

the following summer *Cucurbitaria* spread from the inoculated snags over the whole tree. By April 1931 the whole tree was covered with *Cucurbitaria*, which had produced perithecia as well as pycnidia. In Dec. 1929 four vigorous young trees were transplanted, two to a very wet situation in a bog garden, where their roots were in water, and two to a shady situation. One tree in each situation had many inoculations on it when transplanted, and the other was inoculated after transplanting. The trees in the shady situation



Text-fig. 5. Diagram of the base of a dead snag at the level of the node showing cork barriers in the bark and phloem, and gum in the xylem. *a*, cork; *b*, cork barrier; *c*, cortex; *d*, pericyclic fibres; *e*, phloem; *f*, xylem; *g*, gum in the xylem; *h*, pith. ×20 approx. Bark and phloem exaggerated.

remained normal, but the trees in the bog garden did not come into leaf during the summer of 1930. Thereafter, *Cucurbitaria* spread through the dead trees, and in April 1931 both trees were covered with fructifications, which had also appeared on the main trunk. Other fungi were in evidence on these dead trees, especially *Nectria* and *Fusarium*.

In these cases of spread of *Cucurbitaria* the tree had been killed by the previous treatment, either by cutting it down, or by suffocating its roots in waterlogged soil. *Cucurbitaria* therefore spreads through a tree, which is dead or dying from some other cause. It may, of

course, hasten the dying process, but cannot be regarded as anything but a saprophyte, or at the most a very weak parasite.

In March and June 1930 snags were cut and inoculated on trees other than laburnum to determine whether *Cucurbitaria Laburni* will grow saprophytically on any dead snag, or whether it is capable of utilising only dead laburnum wood. The other trees used were larch, elm, birch, red currant and black currant. The snags died back to the node, but usually fungi other than *Cucurbitaria* fructified on them. However, pycnidia containing large, brown, muriform pycnospores and apparently identical with pycnidia A were found on one elm twig, and on the inoculated surface of one black currant twig. Thus, it is not impossible for *Cucurbitaria* to grow on the dead twigs of other trees, although under natural conditions it would not survive the competition with the normal invaders of the other types of wood.

Summary

1. *Cucurbitaria Laburni* produces perithecia and two types of pycnidia, viz.:

A. Pycnidia with large, brown, muriform pycnospores occur on the twigs and in a few cultures. First year pycnidia of this type contain brown and colourless spores of various kinds.

B. Pycnidia with small, ovate, colourless spores are particularly characteristic of cultures, but sometimes occur on twigs. These probably represent an incompletely developed form of A. The spores are capable of germination.

2. Previous records of various pycnidial types are recorded and discussed.

3. Dead twigs bearing *Cucurbitaria* frequently bear *Phomopsis rudis* also. This was described by Tubeuf as a spore stage of *Cucurbitaria*, but evidence is given here for its being a distinct fungus.

4. *Cucurbitaria Laburni* occurs on the dead branches of laburnum trees in Cambridge, but from the results of inoculations it cannot be regarded as a wound parasite on normal vigorous trees. It fructifies on dead snags, but does not spread unless the tree is seriously weakened by some other cause.

5. The fungus is usually confined to laburnum trees, but inoculations show that it is at least capable of growing on the dead wood of elm and black currant

This investigation was carried out at the Cambridge Botany School, and in conclusion I should like to express my thanks to Mr F. T. Brooks for suggesting and supervising the work, and to Mr E. W. Mason (Imperial Mycological Institute) for his help in identifying certain of the pycnidial stages encountered on laburnums.

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FURTHER NOTES ON BASIDIOMYCETES IN CULTURE

By K. St G. CARTWRIGHT

(With Plates XV and XVI)

The fungi investigated were:

Polyporus fumosus (Pers.) Fr., *P. adustus* (Wild.) Fr. and *Lenzites trabea* (Pers.) Fr. They were grown on 2 per cent. Kepler's malt extract agar.

1. *Polyporus fumosus*

Macroscopic characters.

Type of growth. The mat is at first white cottony, becoming inconspicuously zoned due to the growth being denser near the centre and becoming sodden or more loose and cottony further out. In a mature culture the mat in places becomes slightly coloured a pale cartridge buff but this coloration is never pronounced. The mat is sodden, patchy, appearing slightly farinaceous due to small raised tufts. Finally in cultures six to eight weeks old a smooth raised crust may be formed along the sides of the slant which are in the nature of an incipient hymenial surface. The rate of growth is moderate:

Average rate of growth in mm. per diem

° C.	mm.	° C.	mm.
15	0·25	27	0·64
20	0·51	30	0·15
25	0·74	35	0·0

Fruit bodies. These are formed in two months' old culture in the light as raised, narrow pads cartridge buff in colour (Pl. XVI, fig. 1).

Secondary spore formation. Visible as tufts.

Microscopic characters.

Hyphae. Aerial. These are 1–5 μ in diameter with numerous simple clamps. Branches are often given off from these clamps, which again form clamps, thus giving the appearance that the hyphae are swollen at the septa.

The large hyphae often have two or more clamps at a septum and branches from these may grow downwards parallel to the main hypha, thus giving rise to small strands.

Submerged mycelium. Mostly fine, 1·5–2 μ in width, but large hyphae occur sparingly and these often show irregular swellings and are constricted at the septa.

Clamps are present, though not so numerous as on the aerial mycelium. The mycelium is profusely branched and twisted.

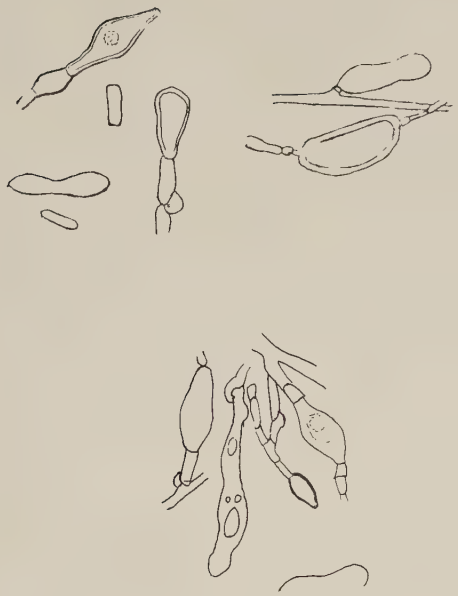

Fig. 1

Fig. 3

Fig. 2

Fig. 4

Clamp connections. Simple, often proliferating.

Secondary spores. Numerous in aerial mycelium, formed in tufts and also scattered. They are mostly terminal, though intercalary ones also occur (Pl. XV, figs. 2 and 3).

Fruit-bodies. These possess a hymenial layer with normal basidiospores (Pl. XV, fig. 4). Secondary spores are present on the mycelium below the hymenial surface.

These spores are pear shaped, oval or more rarely irregular in shape, averaging 8–10 μ, a few as much as 30 μ in length. They are usually formed in from two to three weeks from inoculation.

Crystals. Numerous large rhomboidal crystals occur and in addition a mass of fine crystalline material is formed.

General remarks.

The tufts are formed of very closely woven hyphae which, under low magnification, resemble a hymenial surface with young oval to globose basidia, these under higher magnification being seen to be groups of terminally borne secondary spores (Pl. XV, fig. 2).

The culture somewhat resembles that of a weak culture of *Polystictus versicolor* and is also not unlike that of *Fomes annosus* but without its coloration.

2. *Polyporus adustus*

Macroscopic characters.

Type of growth. The mat is at first dead white, like fine wool or swansdown in texture, and is even throughout the culture. The growing zone is formed by the fine, almost colourless hyphae closely adpressed to the surface of the medium. In the dark there is a great development of woolly, white, aerial mycelium. The mat finally resembles dense swansdown in texture.

Colour range. At first white, changing to pale cartridge buff on ageing.

Rate of growth. Moderate, a boiling tube slant being covered in about eighteen days at 20° C.

Average rate of growth in mm. per diem

° C.	mm.	° C.	mm.
15	0·28	27	0·78
20	0·79	30	0·33
25	0·80	35	0·0

Fruit bodies. Not formed.

Secondary spore formation. Not visible.

Microscopic characters.

Hyphae. Aerial. 2–8·5 μ averaging 3–4 μ in diameter, possessing swellings at septa only where branching is taking place.

Crystal formation not pronounced.

Submerged. Hyphal diameter very variable, 2–9 μ averaging 4–6 μ, with small rod-shaped crystals. Some hyphae show swellings and these develop into chlamydospores, which are moderately thick walled.

Clamps are numerous. The larger hyphae are much vacuolated and septation is frequent. Branching is also frequent.

Clamp connections. Simple clamp connections are present.

Secondary spores. These occur sparingly, mostly on the submerged mycelium. They are generally formed terminally, but some are intercalary. In shape they are oblong or irregular. Size up to 10 μ. They are formed in culture after about three weeks.

Crystals. Small rod shaped.

General remarks.

The culture somewhat resembles a culture of *Daedalea quercina* and has no very definite characters.

Comparison of Polyporus fumosus *and* Polyporus adustus

As these two species of *Polyporus* are somewhat similar in the appearance of their sporophores, it was thought that a comparison of their characters in culture might be of some interest.

From the descriptions just given it will be seen that these two fungi can be readily separated on their cultural characters; they can indeed be distinguished at a glance without the need for microscopic examination (cf. Pl. XVI, figs. 1 and 2). The sparse white mat of *P. fumosus* is entirely different from the somewhat dense mat of swansdown texture formed by *P. adustus*. The differences seen under the microscope are equally as pronounced. In *P. fumosus* secondary spore formation is profuse, whereas in *P. adustus*, although secondary spores are formed sparingly they are not a pronounced feature of the culture and are not formed in tufts on the aerial mycelium as in *P. fumosus*. The large rhomboidal crystals formed in cultures of *P. fumosus* also mark it off from *P. adustus* in which the crystals take the form of small rods.

3. *Lenzites trabea*

Macroscopic characters.

Mat at first white, fluffy, quickly colouring to orange buff, and to capucin yellow, ochraceous buff, and ochraceous salmon. A mature culture shows profuse growth at the top end of a tube-slant, forming a thick, fluffy, brightly coloured cushion. Aerial development gradually becomes less, the lower portion of the slant showing a scant growth of mycelium only faintly tinted yellow or cream.

The depth of coloration is somewhat diluted owing to the growing tips of the aërial hyphae being paler. On removal of these growing tips the depth of coloration is much more pronounced.

Fig. 3

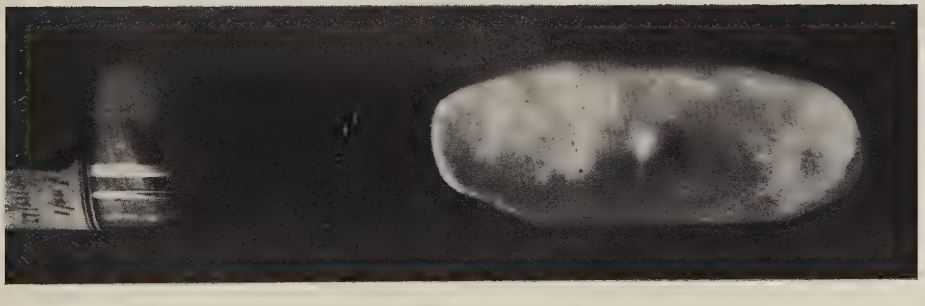

Fig. 2

Fig. 1

Microscopic characters.

Aerial mycelium. Hyaline to yellow in the mass, 1·5–3·5 μ, branching moderately frequent. The hyphae are somewhat irregular in shape. Clamp connections frequent, some very large and proliferating.

Submerged mycelium. Hyaline, 1·5–3·5 μ, branching often at right angles; clamp connections often proliferating.

Fruit bodies. Not formed.

Secondary spores. Abundant, formed both in the aerial and in the submerged mycelium, very irregular in shape, oblong oval to ellipsoidal, and formed terminally and intercalary, 6–40 × 5–10 μ (Pl. XV, fig. 1). Crystals formed but not of value for the identification of the species.

Remarks.

This culture is easy to identify from the macroscopic characters of the mycelial mat and the characteristic colour. The secondary spores are also larger on the average and more irregular in shape than is usual.

A further characteristic is found in its temperature relations, the fungus having a high optimum for growth.

Owing to this its range of distribution appears to be large, as it is not restricted to temperate climates.

It causes a brown cubical rot often in pockets.

The mycelium in the wood shows numerous medallion clamps as well as the simple form, and it is probable that this species takes the place of other species of *Lenzites* in hot countries such as the Sudan.

The culture of *Lenzites trabea* was received through the courtesy of Dr Audrey Richards of Madison.

This culture has been compared with a culture of *L. thermophila* Falck from the Centraal Bureau voor Schimmelcultures, Baarn, with one received direct from Falck (Pl. XVI, fig. 3), and also with a culture kindly sent to us by Dr Liese of *Trametes protracta.*

The four cultures are identical in every respect.

NOTES

SCLEROTIUM RHIZODES AUERSW. IN ENGLAND

In July, 1931, in an old meadow in North Derbyshire, about one mile from Edale Railway Station, certain areas or patches were conspicuous, even at a considerable distance, in which the grass appeared to be bleached, withered or scorched. Examination of the grasses (mainly *Agrostis* spp.) in these patches revealed the presence of numerous sclerotia on them, each about the size of a poppy seed, some of which were whitish and smooth and others black and wrinkled. No fructifications could be found in association with them. The size and appearance of the sclerotia agreed closely with the published descriptions of those of *Sclerotium rhizodes* Auersw., and comparison with continental specimens, kindly made by Miss Dickinson at the Royal Botanic Gardens, Kew, confirmed their identity with this species. This fungus does not appear to have been recorded hitherto in Britain. It was first recorded on the continent in 1849, having been found by Auerswald in Germany, near Leipzig, on *Calamagrostis*. It is now known to be widely distributed and to attack a variety of grasses in many European countries, and it occurs also in the U.S.A., where, in addition to attacking meadow grasses, it has been found occasionally causing damage to wheat.

The field in which the disease was found in England is situated on a hillside about 400 ft. above sea-level, in a district of high rainfall where the land is very poor. The soil overlies Millstone Grit and is very wet and sour, particularly in places where numerous small springs arise.

H. H. STIRRUP.

A RARE RUST ON FUCHSIA

Towards the end of January, 1932, *Fuchsia* leaves attacked by a Rust, from a Cardiff propagating house, were sent to the Ministry of Agriculture's Plant Pathological Laboratory for examination. The Rust was at first confined to cuttings in a few boxes of the variety Golden Treasure, but a fortnight later, when these had become badly affected, others, of two varieties, showed slight attack. The disease was most severe on the lower leaves and ultimately these became discoloured, dried up and fell off.

Affected leaves showed indefinite, pale areas on their upper surfaces, and corresponding areas on the lower sides bore numerous uredosori. The older pustules had burst, exposing the pale orange uredospores, and these mature sori were surrounded by younger ones in many of which the wax-like peridium was still intact, or had opened

by a pore through which the uredospores were being extruded. Eventually the peridium splits, exposing the whole mass of spores, surrounded for a time, at any rate, by its fragments.

This Rust recalled that rather commonly occurring in its uredo-stage in this country on *Circaea lutetiana* (*Pucciniastrum Circaeae* Speg.), and the spore measurements did not differ markedly from those of that species. However, on consulting the literature it was found that in 1918 Arthur and Holway had described *Uredo Fuchsiae* as a new species, occurring in Guatemala on *Fuchsia splendens* Zucc. and *Lopezia hirsuta* Jacq. (*Amer. Journ. Bot.* v (1918), p. 538). So far as is known, this is the only record of this species of Rust up to the present. These authors considered that the Rust was probably a *Pucciniastrum*, and Hiratsuka, on the strength of this, made the combination *Pucciniastrum Fuchsiae* (Arth. & Holw.) Hiratsuka (*Journ. Fac. Agric. Hokkaido Imp. Univ. Sapporo.* xxi (1927), 63). This, however, seems unwarranted, as the uredospore stage alone has been encountered as yet.

The Rust from Cardiff was found to agree so closely with the published description of *U. Fuchsiae* that it is considered referable to that species.

The origin of the present attack is unknown, but it is worth noting that the affected cuttings had been taken from a stock of bedded-out plants that had been observed to shed their leaves prematurely two years previously. It is just possible that the Rust may have been on them then. It would appear worth while to attempt to infect *Fuchsia* with other species of *Pucciniastrum* from hosts belonging to the Onagraceae or Rubiaceae.

ALEX. SMITH AND J. REES.

BOTRYTIS CINEREA ON APPLE ROOTS

Botrytis cinerea is known to attack a wide range of plants and in wet seasons it may cause appreciable damage to cultivated crops. It frequently infects fruit (including apples) and at times is found on the woody parts of fruit trees and bushes, as, for example, in the common Dieback disease of gooseberry bushes, and in a disease of raspberry canes recently reported by Harris.* In 1924 it was found on young apple trees, apparently causing cankers on the stems,† and this year it has been seen on apple roots.

As the occurrence of *B. cinerea* on woody roots is unusual, it may be of interest to describe the conditions under which this fungus became associated with damage to apple roots.

* *East Malling Research Sta. Report for 1928–30*, II, Supplement, p. 136, April, 1931.
† *East Malling Research Sta. Report for 1924*, p. 110, Nov. 1925.

Towards the end of January apple stocks on the East Malling Research Station were lifted for transplanting. In one variety (East Malling No. 7) nearly every stock had one or more main roots dead and showing brown when cut. Some of these dead roots bore sclerotia, the smaller ones being 1–2 mm. in diameter, and rounded, while larger ones were more irregular and up to 4 mm. in length. Other roots bore tufts of *Botrytis cinerea* and conidiophores were growing out from one of the sclerotia. Some of the smaller sclerotia were developing on the thin fibrous roots so that they appeared to be supported on stalks growing out from the main roots; the "stalks" however proved to be small roots.

The following information regarding the stocks was given to me by Mr R. J. Garner. They were one-year-old bedded stocks, removed from the stools as one-year layers in the winter of 1930–1 and bedded out on one of the plots at the East Malling Research Station. They grew well during 1931 and produced stout shoots. Towards the end of October they were lifted, collected into bundles of fifty to a hundred and bedded in the ground. They were apparently quite healthy at that time. The rooting of the young stocks was very good and there were numerous fibrous roots characteristic of this particular stock. When they were again lifted on January 20, 1932, the roots were found to be rotting although the stems were perfectly sound.

This stock was the only one in this condition, although several others had been treated similarly. No. 7, however, had produced more fibrous roots than the rest and a possible explanation of the damage is that the large amount of "fibre" in the "bedded in" bundles retained an excessive amount of moisture, thus favouring attack by *Botrytis cinerea*.

H. WORMALD.

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18. Beaumont, Mr Albert, M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon. (1924.)
19. Bewley, Mr W. F., D.Sc., Experimental and Research Station, Cheshunt, Herts. (1922.)
20. Biffen, Professor Sir Rowland H., M.A., F.R.S., 136, Huntingdon Road, Cambridge. (1899.)
21. Biologist, Plant Research Laboratory, Horticultural Gardens, Burnley, Victoria, Australia. (1921.)
22. Birmingham Natural History and Philosophical Society, c/o G. T. Calvert, Esq., Hon. Librarian, Avebury House, 55, Newhall Street, Birmingham. (1920.)
23. Bisby, Mr Guy R., Ph.D., Manitoba Agricultural College, Winnipeg, Canada. (1921.)
24. Blackman, Professor V. H., M.A., F.R.S., Imperial College of Science, South Kensington, London, S.W. 7. (1900.)
25. Blackwell, Miss Elsie M., M.Sc., Botanical Department, Royal Holloway College, Englefield Green, Surrey. (1917.)
26. Blagden, Mr C. O., 46, Portland Road, Holland Park Avenue, London, W. 11. (1910.)
27. Bloom, Mr James Harvey, M.A., 36, Ryde Vale Road, Balham, London, S.W. 12. (1915.)
28. Bolas, Mr B. D., M.Sc., 20, Cambridge Gardens, Winchmore Hill, London, N. 21. (1924.)
29. Bonn, Germany, Institut für Pflanzenkrankheiten, Nuss-Allee 9. (1931.)
30. Borthwick, Professor A. W., O.B.E., D.Sc., Forestry Department, The University, Aberdeen. (1911.)
31. Boston, The Mycological Club, c/o Miss Jennie F. Conant, 26, Prospect Street, Melrose, Mass., U.S.A. (1906.)
32. Bradshaw, Mr F., M.A., D.Sc., Armstrong College, Newcastle-on-Tyne. (1928.)
33. Braid, Professor K. W., B.A., B.Sc., West of Scotland Agricultural College, 6, Blythswood Square, Glasgow. (1922.)
34. Brazier, Mr E., Ty'n-y-gongl, Love Lane, Stourbridge. (1921.)
35. Brenchley, Mr G. H., B.A., Clare College, Cambridge. (1925.)
36. Brett, Miss M., M.Sc., Northern Polytechnic, Holloway Road, London, N. 7. (1921.)
37. Briant, Mr A. K., B.A., Imperial College of Tropical Agriculture, St Augustine, Trinidad, B.W.I. (1927.)
38. Brierley, Mr W. B., D.Sc., F.R.A.I., F.L.S., Institute of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts. (1919.)

39. Bright, Mr T. B., B.A., Chucks Cottage, Walton-on-the-Hill, Surrey. (1927.)
40. Brisbane, The Director, Bureau of Sugar Experiment Stations, Department of Agriculture and Stock, Queensland, Australia. (1930.)
41. British Museum, The Trustees of, Cromwell Road, South Kensington, London, S.W. 7. (1914.)
42. Briton-Jones, Professor H. R., D.Sc., Ph.D., D.I.C., A.R.C.S., Imperial College of Tropical Agriculture, St Augustine, Trinidad, B.W.I. (1923.)
43. Brooks, Mr F. T., M.A., F.R.S., F.L.S., The Botany School, Cambridge. (1907.)
44. Brown University, Library, East Side Station, Providence, R.I., U.S.A. (1920.)
45. Brown, Professor W., M.A., D.Sc., Imperial College of Science, South Kensington, London, S.W. 7. (1922.)
46. Bruxelles, Jardin Botanique de l'État, c/o M. P. van Aerdschot. (1911.)
47. Bryce, Mr G., D.Sc., Department of Agriculture, Ibadan, Nigeria, W. Africa. (1915.)
48. Buckley, Mr W. D., "Lynmouth," 2, Curzon Street, Slough. (1916.)
49. Buddin, Mr Walter, M.A., Laboratory of Plant Pathology, University of Reading, 7, Redlands Road, Reading. (1921.)
50. Buller, Professor A. H. R., D.Sc., Ph.D., F.R.S., University of Manitoba, Winnipeg, Canada. (1911.)
51. Bunker, Mr H. J., B.A., "Lindau," Hatherden Avenue, Danecourt Road, Parkstone, Dorset. (1925.)
52. Bunting, Mr R. H., F.L.S., Imperial College of Science, Biological Field Station, "Hurworth," London Road, Slough. (1921.)
53. Bunyard, Mr G. N., F.L.S., 25, Bower Mount Road, Maidstone, Kent. (1920.)
54. Burgess, Mr R., M.Sc., British Research Association for the Woollen and Worsted Industries, Torridon, Headingley, Leeds. (1928.)
55. Burr, Mr S., The Agricultural Department, The University, Leeds. (1924.)
56. Butcher, Mr R. W., B.Sc., Ph.D., F.L.S., Tees Laboratory, Barnard Castle, Co. Durham. (1922.)
57. Butler, Mr E. J., C.I.E., C.M.G., D.Sc., M.B., F.R.S., F.L.S., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1920.)
58. Cambridge, The Botany School. (1920.)

59. Carne, Mr W. M., F.L.S., Department of Agriculture, Perth, Western Australia. (1928.)
60. Carr, Professor J. W., M.A., F.L.S., Public Natural History Museum, Wollaton Hall, Nottingham. (1896.)
61. Carrothers, Mr E. N., 145, Stranmillis Road, Belfast, N. Ireland. (1925.)
62. Carter, Miss Nellie, D.Sc., Botanical Department, East London College, Mile End Road, London, E. (1929.)
63. Cartwright, Mr K. St G., B.A., F.L.S., The Red House, Kingston Blount, Oxford. (1913.)
64. Castellani, Sir Aldo, M.D., 33, Harley Street, London, W. 1. (1922.)
65. Cayley, Miss Dorothy M., John Innes Horticultural Institute, Mostyn Road, Merton, Surrey. (1913.)
66. Cejp, Dr Karel, Na Slupi 433, Praha (Prague) II, Czechoslovakia. (1930.)
67. Chapman, Mr A. Chaston, F.R.S., F.I.C., Chemical Laboratories, 8, Duke Street, London, E.C. 3. (1926.)
68. Charles, Mr J. H. V., London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C. 1. (1922.)
69. Chaudhuri, Mr H., M.Sc., Ph.D., University of the Punjab, Lahore, India. (1920.)
70. Cheal, Mr W. F., Gosmoor Lane, Elm, nr Wisbech, Cambs. (1927.)
71. Cheel, Mr Edwin, Botanic Gardens, Sydney, New South Wales, Australia. (1919.)
72. Chesters, Mr C. G. C., Botanical Department, The University, Edgbaston, Birmingham. (1930.)
73. Chona, Mr B. L., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1929.)
74. Ciferri, Dr R., P.O. Box 113, Santiago, Dominican Republic, W.I. (1926.)
75. Cleland, Mr J. Burton, M.D., Professor of Pathology, University of Adelaide, South Australia. (1918.)
76. Clement, Mr E., F.L.S., The Cottage, Yew Tree Road, Southborough, Kent. (1927.)
77. Clouston, Mr D., M.A., B.Sc.(Agr.), Botany Department, King's College, Old Aberdeen. (1931.)
78. Coimbatore, The Principal, Agricultural College, Madras, South India. (1929.)
79. Connecticut Agricultural Experiment Station, New Haven, Connecticut, U.S.A. (1929.)
80. Cook, Mr W. R. I., B.Sc., Ph.D., Department of Botany, The University, Bristol. (1924.)

81. Cooper, Miss Charlotte A., California Lane, Bushey Heath, Herts. (1911.)
82. Cooper, Mrs V. Astley, Clerdoun, Epsom Road, Ewell, Surrey. (1921.)
83. Copenhagen, Universitets-Bibliothek, c/o P. Haase & Son, Løvstraede 8, København K., Denmark. (1923.)
84. Cornell University, The Library, New York State College of Agriculture, Ithaca, N.Y., U.S.A. (1920.)
85. Corner, Mr E. J. H., B.A., F.L.S., Assistant Director, Botanic Garden, Singapore, Straits Settlements. (1924.)
86. Cotton, Mr Arthur D., F.L.S., Keeper, Herbarium, Royal Botanic Gardens, Kew, Surrey. (1902.)
87. Cunningham, Mr G. H., Ph.D., Plant Research Station, Box 240, Palmerston North, New Zealand. (1922.)
88. Curtis, Miss Kathleen M., M.A., D.Sc., D.I.C., F.L.S., Mycologist, Biological Department, Cawthron Institute of Scientific Research, Nelson, New Zealand. (1917.)
89. Cutting, Mr E. M., M.A., F.L.S., Botanical Department, University College, Gower Street, London, W.C. 1. (1920.)
90. Dade, Mr H. A., A.R.C.S., Department of Agriculture, Aburi, Gold Coast, West Africa. (1927.)
91. Darbishire, Professor O. V., B.A., Ph.D., F.L.S., The University, Bristol. (1913.)
92 Das, Mr Kedarnath, C.I.E., M.D., Principal, Carmichael Medical College, 1, Belgachia Road, Calcutta, India. (1922.)
93. Davies, Mr D. W., B.Sc., Adviser in Mycology, Agricultural Buildings, University College of Wales, Aberystwyth. (1923.)
94. Davis, Mr J. Jefferson, B.S., M.D., University of Wisconsin, Madison, Wis., U.S.A. (1921.)
95. Day, Mr E. Metcalfe, Rowan Cottage, Minchinhampton, Glos. (1921.)
96. Day, Mr W. R., B.A., B.Sc., Imperial Forestry Institute, Oxford. (1928.)
97. Dehra Dun, The Forest Botanist, Forest Research Institute and College, U.P., India. (1929.)
98. Deighton, Mr F. C., M.A., Mycologist, Department of Lands and Forests, Freetown, Sierra Leone, West Africa. (1925.)
99. Dickinson, Mr S., Botanical Department, University College, Cardiff, S. Wales. (1921.)
100. Dickson, Mr B. T., B.A., Ph.D., Senior Mycologist, Council for Scientific and Industrial Research, 314, Albert Street, East Melbourne, Australia. (1923.)

101. Dodge, Dr Carroll W., Missouri Botanical Garden, 2315, Tower Grove Avenue, St Louis, Missouri, U.S.A. (1926.)
102. Dowson, Mr W. J., M.A., D.Sc., The Botany School, Cambridge. (1920.)
103. Duncan, Mr J. T., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1. (1930.)
104. East African Agricultural Research Station, Amani, *via* Tanga, Tanganyika Territory, East Africa. (1929.)
105. Edwards, Mr W. H., Belle Vue, Barline, Beer, Devon. (1896.)
106. Elliot, Rev. E. A., Great Longstone, Derbyshire. (1923.)
107. Elliott, Mr W. T., D.D.S., L.D.S., F.L.S., F.Z.S., Arden Grange, Tanworth-in-Arden, Warwickshire. (1913.)
108. Elliott, Mrs J. S. Bayliss, D.Sc. (B'ham), B.Sc. (Lond.), Arden Grange, Tanworth-in-Arden, Warwickshire. (1911.)
109. Ellis, Mr E. H., B.Sc., Department of Botany, British Museum (Nat. Hist.), Cromwell Road, London, S.W. 7. (1924.)
110. Ellis, Miss E. M., St Hugh's College, Oxford. (1930.)
111. Ellis, Mr Holmes, F.R.M.S., 23, Townley Street, Colne, Lancs. (1927.)
112. Emoto, Dr Y., Tokugawa Biological Institute, Ebara-Gun, Tokyo-Fu, Japan. (1929.)
113. Engledow, Professor F. L., M.A., School of Agriculture, Cambridge. (1922.)
114. Essex Field Club, c/o Mr Percy Thompson, F.L.S., Essex Museum of Natural History, Romford Road, Stratford, London, E. 15. (1919.)
115. Exeter, Librarian, University College of the South-West of England. (1926.)
116. Eyre, Miss J. C., Maitlands Cottage, Ipplepen, Newton Abbot, Devon. (1915.)
117. Faulkner, Mr F., c/o Gillanders, Arbuthnot & Co., Bombay, India. (1930.)
118. Fenton, Mr E. W., M.A., B.Sc., F.L.S., Biology Department, Edinburgh and East of Scotland College of Agriculture, Edinburgh. (1920.)
119. Fikry, Mr Amin, Plant Protection Section, Cotton Research Board, Giza, Egypt. (1927.)
120. Findlay, Mr W. P., B.Sc., A.R.C.S., Courte Falaise, Sevenoaks, Kent. (1928.)
121. Finlayson, Mr Raymond A., F.L.S., Official Seed Testing Station, Huntingdon Road, Cambridge. (1910.)
122. Fisher, Mr S. D. P., Sackville Street, Leeds. (1930.)
123. Forwood, Mr R., Wynfield, Parkgate, Cheshire. (1930.)
124. Freemantle, Mr I. S., 7, Ampton Road, Edgbaston, Birmingham. (1931.)

125. Gadd, Mr C. H., D.Sc., Tea Research Institute, Nuwara Eliya, Ceylon. (1921.)
126. Galloway, Mr L. D., 2, Milton Crescent, Cheadle, Cheshire. (1928.)
127. Gardner, Capt. Frederic, c/o Barclays Bank, Jersey, C.I. (1898.)
128. Garside, Mr S., M.Sc., F.L.S., Botanical Department, Bedford College, Regent's Park, London, N.W. 1. (1922.)
129. Gates, Professor R. R., B.Sc., Ph.D., F.R.S., F.L.S., King's College, Strand, London, W.C. 2. (1921.)
130. Gäumann, Dr E. A., Prof. f. spezielle Botanik, Universitätsstrasse 2, Zürich 6, Switzerland. (1928.)
131. Gilbert, Dr E. M., Botanical Department, University of Wisconsin, Madison, Wis., U.S.A. (1922.)
132. Gilbert, M. E., Docteur en Pharmacie, 6, Rue de Laos, Paris (15^{e}), France. (1924.)
133. Glasstone, Mrs V. F. C., B.A. (Oxon.), 15, Northumberland Road, Sheffield. (1930.)
134. Gorman, Mr M. J., A.R.C.Sc.I., Albert Agricultural College, Glasnevin, Dublin. (1925.)
135. Gough, Mr G. C., B.Sc., A.R.C.S., Ministry of Agriculture, Cornwall Buildings, 45, Newhall Street, Birmingham. (1923.)
136. Gould, Mr F. G., Woodrising, Trapps Hill, Loughton, Essex. (1918.)
137. Green, Col. C. Theodore, A.M.S., M.R.C.S., L.R.C.P., F.L.S., 31, Shrewsbury Road, Birkenhead. (1901.)
138. Green, Miss E., M.Sc., 15, Gower Street, London, W.C.1. (1925.)
139. Green, Miss F. Mary, The Botany School, Cambridge. (1931.)
140. Gregor, Mrs M. J. F., Ph.D., Royal Botanic Garden, Edinburgh. (1927.)
141. Gregory, Mr P. H., Ph.D., 16, York Avenue, Hove, Sussex. (1930.)
142. Grieve, Mr B. J., M.Sc., 11, Drayton Gardens, London, S.W. 10. (1931.)
143. Grinling, Mr C. H., B.A., 71, Rectory Place, Woolwich, London, S.E. 18. (1913.)
144. Grove, Miss Jessica H., 14, The Tything, Worcester. (1927.)
145. Gwynne-Vaughan, Professor Dame Helen, G.B.E., D.Sc., LL.D., F.L.S., 93, Bedford Court Mansions, London, W.C. 1. (1906.)
146. Hall, Miss M. P., Plant Pathology Department, Imperial College of Science, South Kensington, London, S.W. 7. (1930.)
147. Hanna, Mr W. F., M.Sc., Dominion Rust Research Laboratory, Agricultural College, Winnipeg, Canada. (1925.)

148. Hansford, Mr C. G., M.A., F.L.S., Mycologist, Department of Agriculture, Kampala, Uganda. (1921.)
149. Harris, Mr R. V., B.Sc., Horticultural Research Station, East Malling, Kent. (1924.)
150. Harrison, Mr T. H., B.Sc. (Agr.), Hawkesbury Agricultural College, Richmond, N.S. Wales, Australia. (1931.)
151. Harvard University, The Library, Cambridge, Mass., U.S.A. (1923.)
152. Hastings, Mr Somerville, M.S., F.R.C.S., 43, Devonshire Street, Portland Place, London, W. 1. (1913.)
153. Heim, M. Roger, Assistant au Muséum d'Histoire Naturelle, 63, Rue de Buffon, Paris (V^{e}), France. (1930.)
154. Heimbeck, Mrs Louise, Brosoe, Levanger, Norway. (1923.)
155. Hemmi, Dr Takewo, Phytopathological Institute, Department of Agriculture, Kyoto Imperial University, Kyoto, Japan. (1923.)
156. Hildyard, Mr F. W., 1, Lichfield Road, Kew, Surrey. (1913.)
157. Hiura, Professor Makoto, Gifu Imperial College of Agriculture, Naka-Mura, Gifu, Japan. (1931.)
158. Hoggan, Miss I. A., Department of Plant Pathology, University of Wisconsin, Madison, Wis., U.S.A. (1923.)
159. Holden, Professor H. S., D.Sc., F.L.S., Botanical Department, University College, Nottingham. (1923.)
160. Honolulu, Association of Hawaiian Pineapple Canners, P.O. Box 3166, Hawaii. (1929.)
161. Honolulu, The Library, Experiment Station, S.P.A., Box 411, Hawaii. (1920.)
162. Hopkins, Mr J. C. F., Agricultural Department, Salisbury, S. Rhodesia. (1930.)
163. Horne, Mr A. S., D.Sc., F.L.S., F.G.S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1921.)
164. Howard, Mr H. J., F.R.M.S., F.L.S., "Lingfield," 6, College Road, Norwich. (1918.)
165. Howarth, Mr W. O., M.Sc., F.L.S., Botanical Department, The University, Manchester. (1927.)
166. Hughes, Mr G. C., Chesterton, Bicester, Oxon. (1898.)
167. Hughes, Mr J. S., M.A., University Observatory, Oxford. (1927.)
168. Hull, The Librarian, Botanical Department, University College. (1929.)
169. Humphrey, Mr C. J., Mycologist and Plant Pathologist, Bureau of Science, Manila, Philippine Islands. (1921.)
170. Hunter, Mrs R., B.Sc., Imperial College of Tropical Agriculture, St Augustine, Trinidad, B.W.I. (1925.)

171. Hurrell, Mr H. E., 25, Regent Street, Great Yarmouth. (1921.)
172. Hurst, Mr C. P., Landulph Rectory, Saltash, Cornwall. (1928.)
173. Imperial College of Tropical Agriculture, Trinidad, B.W.I. (1921.)
174. Iowa, The Library, State University of Iowa, Iowa City, U.S.A. (1923.)
175. Iowa State College, Library, Ames, Iowa, U.S.A. (1927.)
176. Issatchenko, Professor B. L., Directeur du Jardin Botanique, Leningrad, Russia. (1923.)
177. Jaczewski, Professor Arthur de, Director, Institute of Mycology and Phytopathology, Perspective Anglaise 29, Leningrad, Russia. (1922.)
178. John Crerar Library, 86, East Randolph Street, Chicago, Illinois, U.S.A. (1929.)
179. John Innes Horticultural Institute, Mostyn Road, Merton, Surrey. (1924.)
180. Johnson, Mr J. W. Haigh, M.Sc., F.I.C., F.L.S., Walton, Nr Wakefield. (1919.)
181. Johnstone, Miss K. H., Ph.D., Ministry of Agriculture, 10, Whitehall Place, London, S.W. 1. (1927.)
182. Johnstone, Mr R. B., 3, Oswald Gardens, Scotstounhill, Glasgow. (1908.)
183. Jones, Mr G. H., M.A., Plant Protection Section, Ministry of Agriculture, Cairo, Egypt. (1922.)
184. Jones, Mr Robert Fowler, Trinity House, Denton Road, Ilkley, Yorks. (1918.)
185. Jørstad, Mr Ivar, Statsmykolog, Botanisk Museum, Oslo, Norway. (1923.)
186. Kavina, Professor Dr Karel, Professor of Botany, Havlickovy-sady 58, Praha-Kral. Vinohrady (Prague), Czechoslovakia. (1926.)
187. Keissler, Dr Karl, Direktor d. Botanischen Abteilung, Naturhistorisches Museum, Burgring 7, Wien 1/1, Austria. (1924.)
188. Kelly, Dr Howard A., 1418, Eutaw Place, Baltimore, Md., U.S.A. (1921.)
189. Kidd, Mrs Franklin, B.A., The Botany School, Cambridge. (1919.)
190. Klika, Mr Bohumil, Hálkova 37, Prague, Vrsovice 553, Czechoslovakia. (1926.)
191. Knight, Mr H. H., M.A., The Lodge, All Saints' Villas, Cheltenham. (1914.)
192. Knowles, Miss M. C., M.R.I.A., Natural History Museum, Dublin. (1925.)

193. Krieger, Mr L. C. C., Apt. 32, 1300 Massachusetts Avenue, N.W., Washington, D.C., U.S.A. (1921.)
194. Kuala Lumpur, F.M.S., The Director of Agriculture, Straits Settlements, and Federated Malay States. (1930.)
195. Lampitt, Mr L. H., D.Sc., F.I.C., Thornlea, Mount Park, Harrow, Middlesex. (1925.)
196. Leach, Mr R., B.A., Agricultural Department, Zomba, Nyasaland. (1929.)
197. Lee, Miss E. M., 55, Logan Road, Bishopston, Bristol. (1929.)
198. Leicester, The Museum, City of Leicester. (1923.)
199. Lewis, Professor F. J., D.Sc., F.L.S., University of Alberta, Edmonton, Alberta, Canada. (1924.)
200. Line, Mr James, M.A., School of Agriculture, Cambridge. (1921.)
201. Linnean Society, The, Burlington House, Piccadilly, London, W. 1. (1919.)
202. Lloyd Library, The, Cincinnati, Ohio, U.S.A. (1907.)
203. Loader, Miss F. M., Botanical Department, University College, Southampton. (1927.)
204. Lowndes, Mr A. G., M.A., F.L.S., Marlborough College, Marlborough, Wilts. (1922.)
205. Lütjeharms, Herr W. J., Assistent aan's Rijks Herbarium, Leiden, Holland. (1930.)
206. Macdonald, Mr J., 25, Drumsheugh Gardens, Edinburgh. (1929.)
207. Mackenzie, Miss A. D., Research Station, East Malling, Kent. (1921.)
208. Madras University Library, Senate House, Triplicane, Madras, South India. (1925.)
209. Maire, M. René, D.Sc., F.M.L.S., Professeur à la Faculté des Sciences de l'Université, Algiers, Algeria, N. Africa. (1907.)
210. Maltby, Mr G. C., 14, Northwick Road, Evesham. (1923.)
211. Mandalay, Burma, The Principal, Agricultural College. (1930.)
212. Marsh, Mr R. W., M.A., Research Station, Long Ashton, Bristol. (1923.)
213. Martyn, Mr E. B., B.A., Department of Agriculture, Georgetown, British Guiana. (1927.)
214. Mason, Mr E. W., M.A., M.Sc., F.L.S., Imperial Bureau of Mycology, 17, Kew Green, Kew, Surrey. (1921.)
215. Mason, Mrs E. W., Suffield House, Paradise Road, Richmond, Surrey. (1922.)
216. Mason, Mr F. A., F.R.M.S., M.S.P.A., 29, Frankland Terrace, Leeds. (1912.)
217. Massey, Mr R. E., F.L.S., Government Botanist, Department of Agriculture, Khartoum North, Sudan. (1929.)

218. Matthews, Professor J. R., M.A., F.L.S., Botanical Department, The University, Reading. (1921.)
219. McDonald, Mr J., D.F.C., B.Sc., F.L.S., Mycologist, Department of Agriculture, Box 323, Nairobi, Kenya Colony, East Africa. (1923.)
220. McFarland, Dr Frank T., Department of Botany, University of Kentucky, Lexington, Ky., U.S.A. (1924.)
221. McLennan, Dr Ethel I., Botanical Department, Melbourne University, Carlton, Victoria, Australia. (1926.)
222. Mehta, Professor K. C., Ph.D., Department of Biology, Agra College, Agra, U.P., India. (1921.)
223. Metcalfe, Mr C. R., B.A., Ph.D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey. (1926.)
224. Meulenhoff, Dr J. S., Ruychrocklaan 30, The Hague, Holland. (1921.)
225. Michigan Agricultural College Library, East Lansing, Michigan, U.S.A. (1924.)
226. Millard, Mr W. A., D.Sc., Ph.D., The Agriculture Department, The University, Leeds. (1924.)
227. Miller, Professor J. H., B.S., M.S., Ph.D., University of Georgia, Athens, Ga., U.S.A. (1930.)
228. Missouri, The Botanical Garden, St Louis, Mo., U.S.A. (1902.)
229. Mitra, Mr M., M.Sc., Agricultural Research Institute, Pusa, Bihar and Orissa, India. (1928.)
230. Miyabe, Dr Kingo, Professor Emeritus of Botany, Hokkaido Imperial University, Sapporo, Japan. (1919.)
231. Montague, Mrs A., Penton, Crediton, N. Devon. (1898.)
232. Montana Experiment Station, Department of Botany and Bacteriology, Bozeman, Montana, U.S.A. (1930.)
233. Moore, Miss E. S., Ph.D., c/o the Secretary, Department of Agriculture, P.O. Box 994, Pretoria, South Africa. (1923.)
234. Moore, Mr W. C., M.A., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1922.)
235. Morgan, Dr G., Ashley-Hatton, Dyke Road Avenue, Brighton. (1928.)
236. Morris, Mr L. E., Rubber Research Institute, Kuala Lumpur, Federated Malay States. (1924.)
237. Murphy, Professor P. A., Sc.D., A.R.C.Sc.I., M.R.I.A., Department of Plant Pathology, Albert Agricultural College, Glasnevin, Dublin, N.W. 3. (1924.)
238. Murray, Mr G. H., F.E.S., Director of Agriculture, Rabaul, New Britain, Territory of New Guinea, *via* Australia. (1921.)
239. Murrell, Major Percy J., O.B.E., F.R.M.S., "Littlecroft," Orpington, Kent. (1923.)

240. Muskett, Mr A. E., M.Sc., A.R.C.S., Queen's University, Belfast, North Ireland. (1923.)
241. National Collection of Type Cultures, Curator, Lister Institute, Chelsea Gardens, London, S.W. 7. (1921.)
242. National Museum of Wales, Cardiff. (1924.)
243. Nattrass, Mr R. M., B.Sc. (Agric.), Ph.D., Department of Agriculture, Nicosia, Cyprus. (1925.)
244. Nebraska, The Library, University of, Lincoln, Nebr., U.S.A. (1924.)
245. Nederlandsche Mycologische Vereeniging, c/o Mr A. C. S. Schweers, Nassaulaan 17, Alkmaar, Holland. (1920.)
246. New York Botanical Garden, Bronx Park, New York, U.S.A. (1904.)
247. Nicholson, Mr W. E., F.L.S., 50, St Anne's Crescent, Lewes. (1913.)
248. Nitimargi, Mr N. M., M.Ag. (Bombay), Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1930.)
249. Noel, Miss E. F., F.L.S., 37, Burnham Court, Queen's Road, London, W. 2. (1913.)
250. Norman, Mr L. Stafford, Konjeni Estate, Luchenza P.O., Nyasaland. (1927.)
251. North Carolina, Library, University of, Chapel Hill, North Carolina, U.S.A. (1920.)
252. Nursery and Market Garden Industries' Development Society, Ltd., Experimental and Research Station, Cheshunt, Herts. (1922.)
253. O'Connor, Mr P., Ph.D., B.Sc., A.R.C.Sc.I., National Museum, Dublin. (1925.)
254. Ogilvie, Mr L., M.A., M.Sc., Research Station, Long Ashton, Nr Bristol. (1922.)
255. Oke, Mr Alfred William, B.A., F.G.S., F.L.S., 32, Denmark Road, Hove, Sussex. (1908.)
256. Ontario Agricultural College, Library, Guelph, Ontario, Canada. (1920.)
257. Osborn, Professor T. G. B., D.Sc., F.L.S., Botanical Department, The University, Sydney, N.S.W., Australia. (1910.)
258. Ottawa, Ontario, Canada, The Library, Geological Survey. (1926.)
259. Overholts, Dr L. O., Botany Department, Pennsylvania State College, State College, Pa., U.S.A. (1929.)
260. Page, Miss W. M., M.Sc., 19, Ledam Buildings, Bourne Estate, Holborn, London, E.C. 1. (1921.)
261. Park, Mr M., Department of Agriculture, Peradeniya, Ceylon. (1929.)

262. Parke Davis & Co., Medical Research Library, P.O. Box 488, Detroit, Michigan, U.S.A. (1920.)
263. Parker, Mr Theo., The Cooper Field Research Laboratories, Wateringbury, Kent. (1931.)
264. Paul, Mr W. R. C., M.Sc., D.I.C., F.L.S., Department of Agriculture, Southern Division, Galle, Ceylon. (1928.)
265. Paulson, Mr Robert, F.L.S., F.R.M.S., Glenroy, Cecil Park, Pinner, Middlesex. (1918.)
266. Peacock, Dr H. G., The Lawn, Torquay. (1896.)
267. Pearson, Mr Arthur A., F.L.S., 59, Southwark Street, London, S.E. 1. (1911.)
268. Peklo, Dr Jaroslav, Professor of Applied Botany, Bohemian Technical University, Charles Square, Prague II, Czechoslovakia. (1924.)
269. Perthshire Society of Natural Science, c/o Mr James Winter (Hon. Treasurer), 35, George Street, Perth. (1919.)
270. Petch, Mr T., B.A., B.Sc., Stoney Cross, The Chase, King's Lynn, Norfolk. (1911.)
271. Pethybridge, Mr G. H., Ph.D., B.Sc., F.L.S., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1919.)
272. Philadelphia, The Academy of Natural Sciences of Philadelphia, Logan Square, Phil., U.S.A. (1925.)
273. Phillips, Dr H. H., 6, St John's Road, Penge, London, S.E. 10. (1923.)
274. Phillips, Professor J. F. V., B.Sc. (For.), D.Sc. (Edin.), University of the Witwatersrand, Johannesburg, South Africa. (1921.)
275. Ping, Mr A. Wentworth, M.A., "St Olave's," Clifton, York. (1926.)
276. Plowright, Mr C. T. M., B.A., M.B., King Street, King's Lynn. (1901.)
277. Potter, Rev. M. C., Sc.D., M.A., F.L.S., Corley Croft, New Milton, Hants. (1896.)
278. Povah, Dr A. H., Park Naturalist, Yellowstone National Park, Yellowstone Park, Wyoming, U.S.A. (1924.)
279. Preston, Mr N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop. (1920.)
280. Pretoria, South Africa, The Chief, Division of Botany (91403), Department of Agriculture. (1922.)
281. Priestley, Professor J. H., D.S.O., B.Sc., F.L.S., Botanical Department, The University, Leeds. (1912.)
282. Proefstation voor Thee, Departement van Landbouw, Buitenzorg, Java. (1926.)

283. Purdue University, Library, Agricultural Experiment Station, Lafayette, Ind., U.S.A. (1931.)
284. Pusa, Imperial Mycologist, Imperial Agricultural Research Institute, Pusa, Bihar, India. (1921.)
285. Ramsbottom, Mr J., O.B.E., M.A., F.L.S., British Museum, Cromwell Road, South Kensington, London, S.W. 7. (1910.)
286. Ray, Miss Anne, Penarwyn, Gorran Haven, Gorran, Cornwall. (1929.)
287. Rayner, Dr M. Cheveley (Mrs Neilson Jones), Bedford College for Women, Regent's Park, London, N.W. 1. (1921.)
288. Rea, Miss M. W., M.Sc., Salem House, Sydenham, Belfast, Ireland. (1920.)
289. Rees, Mr John, M.Sc., Adviser in Agricultural Botany, University College, Cardiff. (1929.)
290. Reichert, Dr Israel, Plant Pathologist, Palestine Zionist Executive Agricultural Experiment Station, Tel-Aviv, Palestine. (1924.)
291. Rhind, Mr Donald, B.Sc., Economic Botanist, Department of Agriculture, Agricultural College, Mandalay, Burma. (1922.)
292. Rhodes, Miss Mabel, Lister Institute, Chelsea Gardens, London, S.W. 1. (1921.)
293. Rhodes, Rev. P. G. M., D.D., M.A. (Cantab.), F.L.S., St Mary's, Evesham, Worcestershire. (1929.)
294. Richards, Mr R. M., M.B.E., A.R.C.S., F.L.S., The Laboratory, Caledonia Estate, Province Wellesley, Straits Settlements. (1915.)
295. Robson, Mr R., M.Sc., F.Z.S., East Hammingfield, Chelmsford, Essex. (1914.)
296. Roper, Miss Ida M., F.L.S., 4, Woodfield Road, Redland, Bristol. (1921.)
297. Rothamsted Experimental Station, Department of Mycology, Harpenden, Herts. (1923.)
298. Rutgers College and State University of New Jersey, Library, New Brunswick, New Jersey, U.S.A. (1922.)
299. Ryan, Mr G. M., F.L.S., 35, Ladbroke Gardens, London, W. 11. (1923.)
300. Salam, Mr M. M. Abdel-, Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1930.)
301. Salisbury, Professor E. J., D.Sc., F.L.S., Botanical Department, University College, Gower Street, London, W.C. 1. (1921.)
302. Salmon, Professor E. S., F.L.S., South-Eastern Agricultural College, Wye, Kent. (1922.)

303. Sampson, Miss K., M.Sc., University College, Aberystwyth, North Wales. (1920.)
304. Samuel, Mr Geoffrey, The University of Adelaide, South Australia. (1923.)
305. Sanderson, Mr A. R., F.L.S., Austwick Hall, Austwick, Nr Lancaster. (1921.)
306. Sawhney, Mr A., M.Sc., Panjab University, Lahore, India. (1927.)
307. Science Library, South Kensington, London, S.W. 7. (1924.)
308. Scott, Mr W. W., 13, Bishop's Road, Highgate, London, N. 6. (1922.)
309. Searle, Mr G. Odell, B.Sc. (Agric.), Research Botanist, Linen Industry Research Association, Glenmore House, Lambeg, Lisburn, Ireland. (1920.)
310. Seth, Mr N. L., B.Sc. Ph.D., D.I.C., Agricultural College, Mandalay, Burma. (1930.)
311. Sharpe, Mr C. J., Brambleside, Manor Road, Sidcup. (1905.)
312. Sharples, Mr A., A.R.C.S., D.I.C., Mycologist, Department of Agriculture, Kuala Lumpur, Federated Malay States. (1924.)
313. Shaw, Mr F. J. F., D.Sc., A.R.C.S., F.L.S., Imperial Agricultural Research Institute, Pusa, Bihar, India. (1920.)
314. Shear, Dr C. L., U.S. Department of Agriculture, Bureau of Plant Industry, Washington, D.C., U.S.A. (1930.)
315. Small, Mr W., M.B.E., M.A., Ph.D., B.Sc., Director, Department of Agriculture, Zomba, Nyasaland. (1915.)
316. Smart, Miss V., 69, High Street, Littlehampton, Sussex. (1929.)
317. Smith, Mr Alexander, M.A., Ph.D., Ministry of Agriculture, Pathological Laboratory, Milton Road, Harpenden, Herts. (1924.)
318. Smith, Mr F. E. V., B.Sc., Microbiologist, Department of Agriculture, Jamaica, B.W.I. (1922.)
319. Smith, Miss K. E., The Quarry, Lutterworth Road, Nuneaton. (1913.)
320. Smith, Professor Noel J. G., Ph.D., B.Sc., Botany Department, Rhodes University College, Grahamstown, S. Africa. (1924.)
321. South London Botanical Institute, 323, Norwood Road, Tulse Hill, London, S.E. 24. (1921.)
322. Stakman, Professor E. C., University of Minnesota, Department of Agriculture, University Farm, St Paul, Minn., U.S.A. (1922.)
323. Statham, Miss E. M., 2, Westbrook Road, Blackheath, London, S.E. 3. (1926.)

324. Stationery Office, H.M., Superintendent of Publications, Book Dept., Westminster, S.W. 1. (3 subscriptions.) (1920.)
325. Stephens, Miss E. L., B.A., Department of Botany, University of Cape Town, Cape Town, South Africa. (1928.)
326. Stephens, Miss F. L., M.Sc., Department of Botany, British Museum (Natural History), Cromwell Road, South Kensington, London, S.W. 7. (1930.)
327. Stirrup, Mr H. H., M.Sc., Midland Agricultural College, Sutton Bonington, Loughborough. (1922.)
328. Storey, Mr H. H., M.A., Ph.D., East African Agricultural Research Institute, Amani, Tanganyika Territory, East Africa. (1922.)
329. St Paul, Minnesota, U.S.A., The Library, Department of Agriculture, University Farm. (1920.)
330. Sutherland, Mr G. K., M.A., D.Sc., F.L.S., "Bremhill," 21, Combe Park, Bath, Somerset. (1914.)
331. Swansea Field Naturalists' Society, c/o Mr E. R. Brown, 71, Rhyddings Park Road, Swansea, South Wales. (1924.)
332. Swanton, Mr E. W., A.L.S., Educational Museum, Haslemere, Surrey. (1899.)
333. Swedish Academy of Sciences, Royal, Stockholm, Sweden. (1919.)
334. Sydney, Australia, The Librarian, University of. (1922.)
335. Sydow, Herr H., Luitpoldstrasse 33, Berlin, W. 30, Germany. (1931.)
336. Tabor, Mr R. J., B.Sc., F.L.S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1914.)
337. Tagg, Mr H. F., F.L.S., Royal Botanic Garden, Edinburgh. (1921.)
338. Tandy, Mr Geoffrey, B.A., F.L.S., Department of Botany, British Museum, Cromwell Road, South Kensington, London, S.W. 7. (1925.)
339. Taylor, Miss B. K., 98, Cheyne Walk, Chelsea, London, S.W. 3. (1910.)
340. Tennessee, University of, Agricultural Experiment Station, Library, Knoxville, Tennessee, U.S.A. (1926.)
341. Tetley, Miss U., Leyfield, Kirkby Lonsdale, Westmorland. (1929.)
342. Thompson, Miss N. L., B.Sc., 27, Rusholme Road, Putney Hill, London, S.W. 15. (1926.)
343. Tomkins, Mr R. G., M.A., Ph.D., Trinity College, Cambridge. (1925.)

344. United States Department of Agriculture, c/o Wheldon and Wesley, 2–4 Arthur Street, New Oxford Street, London, W.C. 2. (1907.)
345. Vanterpool, Mr T. C., M.Sc., Botanical Department, University of Saskatchewan, Saskatoon, Canada. (1929.)
346. Vasudeva, Mr R. S., Botanical Department, Imperial College of Science, South Kensington, London, S.W. 7. (1929.)
347. Vines, Professor S. H., M.A., D.Sc., F.R.S., F.L.S., Langstone, Exmouth, Devon. (1915.)
348. Wadham, Professor S. M., M.A., Department of Agriculture, The University, Melbourne, Victoria, Australia. (1922.)
349. Wakefield, Miss E. M., M.A., F.L.S., Herbarium, Royal Botanic Gardens, Kew. (1911.)
350. Waldie, Mr J. S. L., B.Sc., C.D.A., Department of Agricultural Botany, The University, Reading. (1925.)
351. Wales, University College of, Librarian, Botanical Department, Aberystwyth, North Wales. (1927.)
352. Wallace, Mr G. B., B.Sc. (Agric.), Ph.D., Department of Agriculture, Morogoro, Tanganyika Territory, East Africa. (1928.)
353. Wallace, Mrs G. B., B.Sc., Morogoro, Tanganyika Territory, East Africa. (1924.)
354. Wallis, Mr A., Westacre, Station Road, Kettering. (1921.)
355. Ware, Mr W. M., M.Sc., South-Eastern Agricultural College, Wye, Kent. (1924.)
356. Washington, Library, State College of Washington, Pullman, Wash., U.S.A. (1924.)
357. Waterhouse, Miss G. M., B.Sc., 35, Southdean Gardens, London, S.W. 19. (1927.)
358. Watson, Mr W., D.Sc., A.L.S., Taunton School, Taunton. (1923.)
359. Welsford, Miss E. J., The Laboratory, Dell Park, Englefield Green, Surrey. (1924.)
360. Westerdijk, Professor Johanna, Javalaan 4, Baarn, Holland. (1923.)
361. Weston, Mr W. A. R. Dillon, M.A., School of Agriculture, Cambridge. (1923.)
362. Whetzel, Professor H. H., M.A., New York State College of Agriculture, Cornell University, Ithaca, N.Y., U.S.A. (1914.)
363. Whitaker, Mr F. Owen, 45, Elmdene Road, Plumstead, London, S.E. 18. (1921.)
364. Whitehead, Mr T., D.Sc., A.R.C.S., University College of North Wales, Bangor. (1920.)
365. Wilkins, Mr W. H., M.A., Department of Botany, The University, Oxford. (1928.)

366. Williams, Mr P. H., 4, Belmont Villas, Windmill Lane, Cheshunt, Herts. (1930.)
367. Williamson, Mrs H. S., B.Sc., 3, Verulam Buildings, Gray's Inn, London, W.C. 1. (1921.)
368. Wilson, Miss A. P., M.B.E., A.R.C.S., 116, Fellows Road, N.W. (1929.)
369. Wilson, Mr Malcolm, D.Sc., A.R.C.S., F.L.S., Royal Botanic Garden, Edinburgh. (1921.)
370. Wiltshire, Mr S. P., M.A., Imperial Mycological Institute, Ferry Lane, Kew, Surrey. (1920.)
371. Wisconsin, The Library, University of, Madison, Wis., U.S.A. (1923.)
372. Wolf, Mr B. L., N.D.A., Cornwall Buildings, 45, Newhall Street, Birmingham. (1923.)
373. Woodcock, Mr A. J. A., M.Sc., F.E.S., Clifton Manor, York. (1926.)
374. Woodward, Mr R. C., Ph.D., School of Rural Economy, Oxford. (1924.)
375. Woolhope, The Naturalists' Field Club, Hereford, c/o Col. M. J. G. Scobie, 2, Offa Street, Hereford. (1896.)
376. Worcestershire Naturalists' Field Club, c/o Mr W. J. Else, Victoria Institute, Worcester. (1921.)
377. Wormald, Mr H., D.Sc., A.R.C.S., Research Station, East Malling, Kent. (1921.)
378. Wright, Mr E. Barton, M.Sc., Scottish Society for Research in Plant Breeding, Craig's House, Corstorphine, Midlothian. (1926.)
379. Yale University, Library, New Haven, Connecticut, U.S.A. (1930.)
380. Young, Miss Elaine M., Ph.D., M.Sc., University of the Witwatersrand, Johannesburg, South Africa. (1927.)
381. Zundel, Dr G. L. I., Botany Building, Pennsylvania State College, State College, Pa., U.S.A. (1929.)
382. Zürich, Switzerland, Botanical Garden and Museum, c/o Dr A. U. Däniker. (1921.)

RULES

Society's Name and Objects

1. The Society shall be called "The British Mycological Society," and its object shall be the study of Mycology in all its branches.

Members of Society

2. The Society shall consist of Honorary Members, Foundation Members and Ordinary Members; the number of Honorary Members shall be limited to 20, and that of Foundation Members to 100, but the number of Ordinary Members shall be unlimited.

Honorary Members

3. Honorary Members shall be persons of pre-eminence in Mycology, or who have rendered special service to the Society.

Foundation Members

4. Foundation Members shall be those Members or Societies who joined the Society previous to the limit of 100 Members having been attained.*

Officers

5. The Officers of the Society shall consist of a President, one or more Vice-Presidents, Treasurer, Secretaries, and Editor or Editors. They shall be elected annually, at the Annual General Meeting of the Society.

Government of Society

6. The government of the Society shall be vested in a Council, which shall consist of the President and other Officers for the time being, together with two or more other Members who shall be elected annually at the General Meeting, and one-half of whom shall retire each year and not be eligible for immediate re-election. The Members to retire shall be those who have been longest in office or, in case of equality, shall be determined by ballot. Ex-Presidents are *ex-officio* Members of the Council.

Every Meeting of the Council shall be duly summoned by the Hon. Secretary by at least seven days' notice in writing to each Member of the Council.

Period of Office

7. The Officers and Council shall hold office as from the 1st of January following their election.

* The limit of 100 Foundation Members was reached 22nd October, 1903.

Plant Pathology Committee

8. The special interests of Plant Pathology shall be delegated to an executive committee, to be called the Plant Pathology Committee of the British Mycological Society. This Committee shall consist of the President and Secretaries *ex-officio* and twelve other members of the Society. The latter shall be elected annually at the Annual General Meeting of the Society, and one-quarter shall retire in rotation each year and shall not be eligible for immediate re-election. The members to retire shall be those who have been longest in office, or, in case of equality, shall be determined by ballot.

The Officers shall consist of a Chairman and a Secretary, to be elected by the Committee each year.

At least two meetings shall be held every year, six members to form a quorum.

The Committee shall have power to appoint for any special purpose a sub-committee consisting either wholly or partly of members of the Committee.

Election of Members

9. Honorary Members shall only be elected at a Meeting of the Society by a majority of the Members then present.

All Ordinary Members shall be proposed and seconded respectively by existing Members, who shall sign a certificate (see Appendix) of recommendation, one at least of the proposers so certifying from personal knowledge. Every candidate for election shall sign an undertaking to abide by the Rules if elected (see Appendix). They shall be elected by a majority of the Members present at any meeting of the Society or by the President and Officers of the Society.

Subscription

10. All Ordinary Members and Societies shall pay an annual subscription of one pound, and Foundation Members five shillings, due on the 1st of January in each year. Honorary Members shall be exempt from any annual subscription.

Any Member wishing to retire from the Society shall give notice to the Hon. Secretary or Treasurer in writing before the 1st of December of the previous year.

Meetings

11. The Society shall hold one or more meetings annually, at a place and time determined by the Members at the preceding Annual General Meeting, or by the Council. The Annual General Meeting, for the election of officers and the transaction of other business shall coincide with the Autumn Foray.

Accounts

12. At the Annual General Meeting of the Society in each year the Hon. Treasurer shall present duly audited accounts.

Alteration of Rules

13. The Rules shall not be altered except by a two-thirds majority of the Members present at an Annual General Meeting. A printed copy shall be sent to every Member of the Society on election, and in the event of alterations to all Members.

APPENDIX

Form of proposal for Ordinary Membership of the British Mycological Society

.....

of.....

.....

being desirous of becoming an Ordinary Member of the British Mycological Society, we, the undersigned Members of the Society, certify that we consider h to be a desirable Member of the Society, and beg to recommend h for election.

Dated this day of 19

..... (From personal knowledge.)

Certificate to be signed by the Candidate

I hereby certify that I desire to become an Ordinary Member of the British Mycological Society and that I will abide by the Rules if elected.

.....

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